National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

NL-339 (r. 88/04)

Canada

THE UNIVERSITY OF ALBERTA

THE EFFECTS OF CHROMIUM LOADING ON EARTHWORMS IN AN AMENDED SOIL

(C)

BY
RUTH BARBARA THERESA HALL

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE
IN
SOIL CHEMISTRY

DEPARTMENT OF SOIL SCIENCE

EDMONTON, ALBERTA
FALL, 1988

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-45634-5

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR:	RUTH BARBARA THERESA HALL
TITLE OF THESIS:	THE EFFECTS OF CHROMIUM LOADING ON EARTHWORMS IN AN AMENDED SOIL
DEGREE:	MASTER OF SCIENCE
YEAR THIS DEGREE GRANTED:	FALL, 1988

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts may be printed or otherwise reproduced without the author's written permission.

(SIGNED) Ruth B Hall

PERMANENT ADDRESS:

#302 4920 54th Ave.

YELLOWKNIFE, N.W.T. X1A 1H7

DATED 05/05/1988

THE UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled THE EFFECTS OF CHROMIUM LOADING ON EARTHWORMS IN AN AMENDED SOIL submitted by RUTH BARBARA THERESA HALL in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in SOIL CHEMISTRY.

M. J. Dudas
Supervisor

Byron Kratochvil

Date: July 26, 1988

ABSTRACT

Chromium occurs naturally in the terrestrial environment in a trivalent and a hexavalent form. Cr(III) and Cr(VI) compounds are used in numerous industrial processes. As a result, large amounts of Cr are entering the environment as waste. Much of this material is applied to soil or disposed of in landfill sites. While the toxicity of Cr to plants, animals and humans has been documented, little is known about the short and long term effects of Cr on soil fauna and processes. Earthworms (*Eisenia fetida*, a readily available, common, geophagous species) were chosen for this study because they are important in many food chains and they are currently being used in wasteland reclamation. The experiments were designed to determine if Cr is accumulated by *E. fetida* and to examine the influence of Cr oxidation state, application level and length of exposure to Cr on this accumulation and on earthworm activity. All treatments were incubated under controlled temperature and light conditions. Cr was measured in soil, earthworms and in H_2O and K_2HPO_4 extracts by a variety of methods.

The experiments clearly show that soil-applied Cr is accumulated by *E. fetida*. Accumulation of Cr is greater from the Cr(III) treatments than from the Cr(VI) treatments. Increasing the level of applied Cr(III) does not affect accumulation of Cr by *E. fetida* but increasing the level of Cr(VI) does. Increased length of exposure to Cr increases accumulation for both Cr(III) and Cr(VI) treatments. In this soil, applied Cr(III) is toxic to *E. fetida* at levels at or above 1500 ppm while applied Cr(VI) is toxic at 75 ppm. Respiration and mineral N levels are adversely affected by the application of either Cr(III) or Cr(VI). The effects of Cr(III) on the microbial population are ameliorated by the presence of earthworms. Both Cr(III) and Cr(VI) adversely affect the activity of *E. fetida* although the effect of Cr(III) is much less than that of Cr(VI). The production of casts and cocoons is negatively affected by the presence of either form of Cr. Earthworm biomass also appears to be reduced over time in the presence of either Cr(III) or Cr(VI). In all cases the effect of Cr(VI) on *E. fetida* is more pronounced than that of Cr(III).

ACKNOWLEDGEMENTS

I would like to acknowledge the financial assistance of the National Science and Engineering Research Council and the Government of the Province of Alberta.

I would like to acknowledge my advisor, Marvin J. Dudas, for his supervision and financial support of this research, and thank the members of my committee, Steve Pawluk and Ron Kratochvil, for their constructive contribution to this work. I would also like to thank Professors W.B. McGill and J.A. Robertson for their ideas and encouragement.

I received considerable technical assistance from numerous people both within and without the department; to all of them: thank you. Mike Abley, Laura Toerper and Park Yee, in particular, made the day to day lab work flow more smoothly.

Many graduate students have also given me help, both physical and otherwise. Dave Burton, Karen Haugen-Kozyra and Mike Rutherford spent much time helping me with various lab techniques and discussing ideas as friends and colleagues. Of course all of F.O.P. contributed to sanity preservation in ways too numerous to mention, as did family and friends as far flung as Thunder Bay, Manhattan (Kansas, not New York) and San Fransisco.

Finally, two individuals in particular have been good friends to me through thick and thin; without them much else other than this thesis would never have been accomplished. To Mike Power and Graeme Spiers, a truly heartfelt thank you!

TABLE OF CONTENTS

ABSTRACT iv
ACKNOWLEDGEMENTS v
TABLE OF CONTENTS vi
LIST OF TABLES viii
LIST OF FIGURES ix
CHAPTER ONE: INTRODUCTION 1
CHAPTER TWO: LITERATURE REVIEW 3
2.1 Some Aspects of Chromium Chemistry 3
2.1.0 Introduction 3
2.1.1 Chromium Soil Chemistry 4
2.1.3 Plants and Chromium 9
2.1.4 Chromium and Microbial Populations 10
2.2 Earthworm Ecology 12
2.2.1 Classification of Earthworms 12
2.2.2 Basic Requirements of Earthworms. 14
2.3 Earthworms and Heavy Metals 18
2.3.1 Uptake and Excretion of Heavy Metals 19
CHAPTER THREE: MATERIALS AND METHODS 26
3.1 Choice of Soil and Earthworm Species 26
3.2 Physical Set-up of Experiments 28
3.3 Outline of Experiments 29
3.3.1 Preliminary Experiment 29
3.3.2 Tolerance trial 31
3.3.3 Earthworm - Cr Interaction Experiment 32
3.3.4 Adsorption Experiment 33
3.4 Sampling Procedures and Laboratory measurements 34
3.4.1 Sampling Procedures 34
3.4.2 Biomass Estimation 35
3.4.3 Solution Chemistry 36
3.5 Cr-Sc Ratio Calculations 36
3.6 Data Analysis 39

CHAPTER FOUR: RESULTS AND DISCUSSION.....40
4.1 Chemistry, Mineralogy and Adsorption.....40
4.2 Accumulation of Cr in Eisenia fetida.....42
4.3 Effects of Chromium on Earthworm Activity.....57
4.4 Effects of Eisenia fetida on Chromium Chemistry.....69
CHAPTER FIVE: SUMMARY AND CONCLUSIONS.....72
CHAPTER SIX: REFERENCES.....76
APPENDIX: ADDRESSES OF EXTERNAL ASSISTANCE.....87

LIST OF TABLES

Table 1: Chemistry and mineralogy of 'Didsbury' soil 41
Table 2: ANOVA results for the variables respiration and mineral N.....60
Table 3: Means for various solution Cr parameters.....70

LIST OF FIGURES

Figure 1: Schematic of Cr mechanisms in soil 6
Figure 2: Flow of heavy metals through earthworms 22
Figure 3: Experimental columns 30
Figure 4: Measured Cr in solution *vs.* applied Cr(III) and Cr(VI) 43
Figure 5: CF ratios for E1 treatment 45
Figure 6: CF ratios for E2 treatment 46
Figure 7: Total Cr in worms *vs.* Incubation (E1-Cr(VI) treatment) 48
Figure 8: Total Cr in worms *vs.* Incubation (E2-Cr(VI) treatment) 49
Figure 9: Total Cr in worms *vs.* Incubation (E1-Cr(III) treatment) 50
Figure 10: Total Cr in worms *vs.* Incubation (E2-Cr(III) treatment) 51
Figure 11: Total Cr in worms *vs.* Soil applied Cr 53
Figure 12: "Percent Kill" *vs.* Soil applied Cr 54
Figure 13: Respiration for E1, E2 and NW tratments, within Cr treatments 61
Figure 14: Changes in respiration over time for all treatment combinations 63
Figure 15: Total unfumigated N for E1, E2 and NW treatments, within Cr treatments 65
Figure 16: Changes in total unfumigated N over time for all treatment combinations 67

CHAPTER ONE: INTRODUCTION

The significance of earthworms in the development and maintenance of a wide variety of soil properties and processes has only recently been widely recognized (Bouché, 1985) and information is still fragmentary in many aspects (Lee, 1985). Nonetheless some species of earthworms have been studied for their potential use in sewage sludge treatment and disposal (Mba, 1983; Hatanaka *et al.*, 1983; Hartenstein *et al.*, 1981a; Southwell and Maier, 1981) and are currently used in waste disposal in Japan (Nakamura, 1982). Various workers have considered the colonization (both natural and man assisted) by earthworms of reclaimed mine spoil sites (Rushton, 1986; Curry and Cotton, 1983; Pietz *et al.*, 1984). Earthworms have also been considered as bioindicators of soil polluted with PCB's (Kreis *et al.*, 1987) and heavy metals (Martin and Coughtrey, 1982; Helmke *et al.*, 1979).

Chromium has not usually been one of the heavy metals studied when examining earthworm - metal interactions. This is perhaps an unfortunate oversight. Chromium is used in a wide variety of industrial processes, including petroleum refining, electric power generation, pulp and paper manufacture, and leather tanning. It is perhaps the most ubiquitous of industrial metals (Adriano, 1986; NRCC, 1976). As a result, levels of Cr in industrial waste often exceed background levels by one or more orders of magnitude. In Canadian soils Cr levels range from 2.5 to 100 ppm with an average of 43 ppm (McKeague and Wolynetz, 1980). Cr in fertilizers and in industrial waste material can range from trace amounts to 4000+ ppm depending on the industrial process involved (Adriano, 1986; NRCC, 1976). Where levels of Cr are high relative to background a potential health and environmental hazard may exist. While the toxicity of Cr to plants, animals and humans has been documented (Jaworski, 1984; NRCC, 1976) little is known about the short and long term affects of Cr on soil fauna.

As consumers of soil and surface detritus, earthworms and other soil invertebrates will be exposed to terrestrially deposited heavy metals. The reduced activity or absence of earthworms could have significant effects on the soil. Recent work in the Canadian prairies suggests that in some soils the presence of earthworms may determine the meso and microstructure (S. Pawluk, personal communication; Shaw and Pawluk, 1986b). Earthworms

increase macroporous infiltration and as such may be important in reducing surface runoff (Zachmann *et al.*, 1987; Kladivko *et al.*, 1986). Both the mixing of litter (Scheu, 1987c) and the rate of decomposition of that litter are adversely affected by a lack of, or reduction in earthworms (Scheu, 1987b; Shaw and Pawluk, 1986a; Mitchell *et al.*, 1982; Reichle, 1977). If heavy metals are retained by earthworms they may be passed up the food web. Predation of earthworms is common to many animals (Macdonald, 1983).

Given the increasing interest in and use of earthworms in reclamation, and the increasing amounts of Cr in industrial landfill, this study was designed to examine some aspects of earthworm - Cr interactions. The objectives of the study were as follows:

1. To determine if *Eisenia fetida* accumulates chromium.

2. To determine if Cr accumulation by *E. fetida* varies with;
   a) the oxidation state of Cr (Cr(III) versus Cr(VI)),
   b) the level of Cr application,
   c) the length of exposure to Cr.
3. To determine at what application levels Cr becomes toxic.

4. To determine if the type and form of Cr influences earthworm activity.

5. To determine if the effects of Cr on *E. fetida* are different if there is a time lag (1 to 4 days) between application of Cr to the soil and the introduction of earthworms.

CHAPTER TWO: LITERATURE REVIEW

This review considers some general aspects of earthworm ecology and of chromium chemistry in light of the present study and more specifically examines the current literature concerning the interaction of earthworms and heavy metals. It is by no means exhaustive,but attempts to discuss that body of literature most directly concerned with the objectives of the present study, and to provide the context in which the work was undertaken. The discussion is divided into three sections. The first considers chromium chemistry as it relates to soil systems. The second section considers earthworm ecology and examines the effects of earthworms on various soil properties and processes. The third section considers the interactions of earthworms with heavy metals and where possible focuses on the literature dealing with earthworms and chromium.

2.1 Some Aspects of Chromium Chemistry

2.1.0 Introduction

Chromium is the 24th element of the periodic table, with an atomic weight of 51.996. It is never found as the pure metal but may take oxidation states from -2 to +6. Of these only five are found in nature: 0,+3 and +6 are common, while +4 and +5 are transient intermediaries. In the zero oxidation state (metallic Cr) Cr is resistant to oxidation and is used to form corrosion resistant alloys with other metals. In the trivalent state, Cr forms extremely stable complexes with both organic and inorganic compounds. This property is exploited both biologically and industrially where Cr (III) compounds are used as chemical and enzymatic stabilizers. The hexavalent form of Cr is highly mobile, usually anionic and a strong oxidant (E° = +1.33 for Cr(IV) fi Cr(III)). It is rare, reactive and is used as an oxidant and chemical stabilizer.

The industrial value of Cr is derived from the oxidizing power of Cr(VI) and the chemical stability of Cr(0) and Cr(III) compounds. These characteristics are also what makes Cr both a necessary mammalian micronutrient and a powerful toxin. Cr(III) combines with glutathione and dinicotinic acid to form the Glucose Tolerance Factor (GTF) (Korallus, 1986;

Jaworski 1984; Mertz, 1979,1969). GTF is readily absorbed across the gut wall into the bloodstream where it acts to bind insulin to the membrane of fat cells. This stimulates the cell to absorb glucose from the bloodstream to form glycogen (Jaworski, 1984; Mertz, 1969). Cr(III) deficiency symptoms closely resemble those of diabetes mellitus. However, excessive doses of Cr(III) can have adverse affects. Cr(III) has a high affinity for ATP and will form Cr-ATP complexes which then compete with Mg-ATP as substrate for enzymes. Cr-enzymes are inoperative. Non-specific binding of enzymes and hormones by Cr(III) also occurs (Jaworski, 1984; NRCC, 1976). There is no proven biological function for Cr(VI) (Hartford, 1986; Jaworski, 1984; Mertz, 1979). Unlike Cr(III), Cr(VI) in its common forms can diffuse across cell membranes. This is largely due to its small size and anionic nature. Once inside a cell, Cr(VI) is reduced to Cr(III). Either during or subsequent to this reduction Cr acts as an enzyme poison by stabilizing compounds in incorrect configurations. Due to the stability of reacted Cr(III), these enzymes are permanently poisoned (Jaworski, 1984; Mertz, 1979; NRCC, 1976). Cancer, respiratory and kidney problems and skin reactions can result from exposure to very low doses of Cr(VI). Exposure to airborne Cr(VI) at levels equal to or lower than 0.05 mg/m^{-3} resulted in respiratory problems (NRCC, 1976). Cr(VI) is also a potential contaminant of lakes, rivers and groundwater. Fish are extremely sensitive to both Cr(VI) and Cr(III) as are some other aquatic species (Jaworski, 1984; NRCC, 1976).

2.1.1 Chromium Soil Chemistry

Until recently research concerning Cr concentrated on the human health problems related to: a) industrial exposure to Cr(VI) compounds and b) Cr(III) deficiency. Cr in the soil system was generally assumed to be in the Cr(III) oxidation state (or readily reduced to it) and both insoluble and immobile (Bartlett and Kimble, 1976; Breeze, 1973). The effects of Cr of both oxidation states on various aspects of the plant-microbial-mineral soil system were less well known.

The mobility and availability of Cr in soil is governed by a variety of processes. Rai *et al.* (1988, 1987, 1986) have recently conducted a series of studies looking at Cr in the soil-geological system particularly with respect to

waste from the electrical utilities industry. In Figure 1 the major factors and processes influencing Cr are shown schematically. The major processes are redox transformation, precipitation and dissolution and adsorption/desorption. These processes are modified by the presence of other ions, pH and Eh, and the amount and type of organic matter present. The chemistry of each oxidation state will be considered in light of these environmental factors and processes.

The solubility of Cr(III) decreases as pH increases, with almost complete precipitation at pH 5.5 (Bartlett and Kimble, 1976; James and Bartlett, 1983a). Availability is governed almost exclusively by precipitation/dissolution processes, and specifically by the solubility of $Cr(OH)_3$ and $Cr_xFe_{1-x}(OH)_3$ (Rai *et al.*, 1986). Precipitation kinetics for $Cr(OH)_{3(ss)}$ are rapid, and it exhibits amphoteric behaviour, with minimum solubility occurring between pH 6 and 10.5, at levels less than the U.S. Environmental Protection Agency drinking water standard of 1 ppm. The mole fraction of Cr present as $Cr(OH)_{3(ss)}$ controls the aqueous concentration of Cr in equilibrium with $Cr_x Fe_{1-x}(OH)_{3(ss)}$ (Rai *et al.*, 1986). The solubility of $Cr_x Fe_{1-x}(OH)_{3(ss)}$ is lower than that of $Cr(OH)_{3(ss)}$, resulting in extremely low concentrations of Cr(III) present under acidic conditions (pH 2 - 6). The presence of sufficient $Fe(OH)_3$ further reduces the solubility of $Cr_x Fe_{1-x}(OH)_{3(ss)}$. Precipitation /dissolution of Cr(III) is marked as A on Figure 1.

The presence of ions other than Cr, amorphous oxides, and organic compounds will also alter the solubility and availability of Cr(III). Amorphous manganese oxides have been shown to alter Cr(III) solubility by oxidation of Cr(III) to Cr(VI) (Bartlett and James, 1979; Grove and Ellis, 1980; Eary and Rai, 1987). Eary and Rai (1987) postulated the following mechanism for the oxidation of Cr(III) by ßMnO_2:

$$Cr(III) + 1.5\ MnO_2 + 6H^+ \rightarrow Cr(VI) + 1.5Mn(II) + 3H_2O \qquad (1)$$

They found that Cr(III) oxidation occurred from pH 3 to 10 although the amount oxidized greatly decreased above pH 5. This wide range of pH over which oxidation occurred was not predicted from Cr(III) solubility data. Thus, over extended time periods, with stable redox conditions and a lack of effective reductants for Cr(VI) considerable more Cr(VI) could be in solution than otherwise predicted.

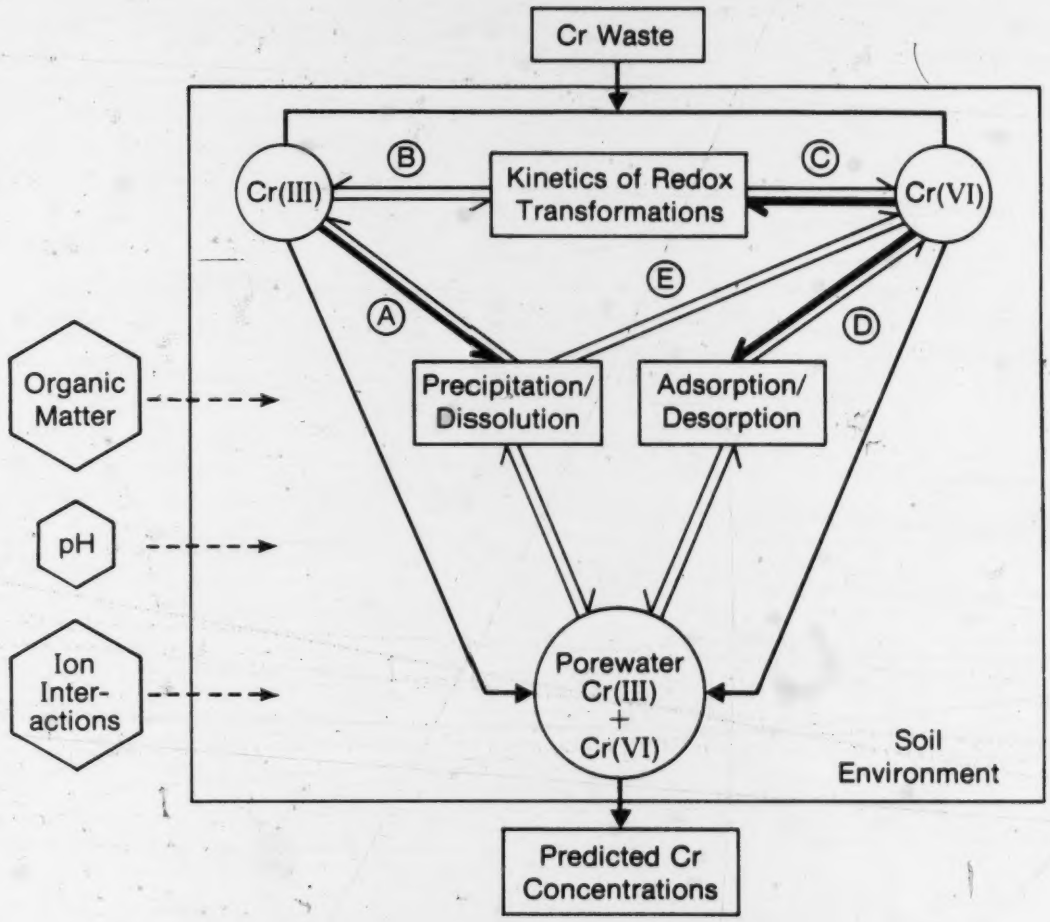


Figure 1. Schematic drawing of dominant Cr mechanisms and of Cr behaviour in the soils/geologic environment. A to E indicate processes described in the text. Heavy arrows are dominant processes. Hexagons and dashed lines indicate factors which alter the soil environment and therefore affect the processes shown. (adapted from Rai et al., 1986)

Cr(III) oxidation was found to be affected by the surface area of MnO_2. An order of magnitude increase in the surface area of MnO_2 resulted in an equivalent increase in Cr(VI) produced (Rai *et al.*, 1986). The rate of Cr(III) oxidation is relatively independent of pH, initial chromium concentration and O_2 levels. In an attempt to explain these results, Rai *et al.* (1986) calculated and compared the ratios of the products of Equation (1), Mn(II)/Cr(VI). High ratios (Mn(II) larger) were interpreted as indicating that acidic dissolution of ßMnO_2 was the dominant process. Low ratios (~2.0 or less) were interpreted as indicating Cr(III) oxidation was dominant. High initial Cr(III) produced low ratios as did increasing pH. Based on these results, Eary and Rai (1987) proposed that Cr(III) competes for reactive sites on the MnO_2 surface that would otherwise be involved in acid MnO_2 dissolution. They also suggested that surface Mn(IV) is the oxidizing agent for Cr(III), and not merely a catalyst for oxidation of Cr(III) by dissolved oxygen. For Cr(III) oxidation by ßMnO_2 (Equation 1) Rai *et al.* (1986) proposed the following three step process:

1. Adsorption of Cr(III) onto favourable oxidation sites on the MnO_2 surface.
2. Oxidation of adsorbed Cr(III) to Cr(VI) by surface Mn(IV).
3. Desorption of reaction products, Cr(VI) and Mn(III).

The third step was thought to be the rate limiting step. In acid pH conditions, the dominant form of Cr(III) is the $CrOH_2^+$ cation while the dominant form of Cr(VI) is the $HCrO_4^-$ anion. The zero point of charge for ßMnO_2 occurs at pH 7.3(±0.2) Rai *et al.* (1986), giving ßMnO_2 a very strong positive surface charge under acid pH conditions. With such a positive surface charge, $HCrO_4^-$ will be held much more strongly to the MnO_2 surface than $CrOH_2^+$. Thus the rate limiting step under acidic conditions is likely the desorption of Cr(VI) and Mn(III) from the MnO_2 surface. The occupation of surface sites by Cr(VI) will also directly decrease the availability of new reactive sites for Cr(III) adsorption (step 1). Under alkaline conditions, $Cr(OH)_{3(ss)}$ will reduce Cr(III) concentration and therefore cause step 1 to be rate limiting. Oxidation of Cr(III) to Cr(VI) is marked B in Figure 1.

Pyrolusite (ßMnO_2) is rare in soils although it is the most studied of manganese oxides (McKenzie, 1977). Rai *et al.* (1986) chose ßMnO_2 for their studies because it is the most chemically pure and crystalline form of MnO_2.

It will therefore have a lower surface energy (and higher zero point of charge) than other more common forms of MnO_2. Oxidation of Cr(III) to Cr(VI) by manganese oxides is therefore likely to be more rapid in soils than shown by Rai *et al.*(1986). Rai *et al.* (1986) worked with pure ßMnO_2/Cr systems, thus eliminating most possible interferences. The presence of both Mn(II) and other heavy metals on the oxide surface will inhibit Cr(III) oxidation by occupying adsorption sites (Bartlett, 1986). Soluble organic complexes can both help and hinder Cr(III) oxidation. James and Bartlett (1983b) found that increased amounts of simple organics resulted in both increased solubility of Cr(III) complexes and increased oxidation of Cr(III). Diffusion of Cr(III) through the soil as soluble Cr-organic complexes may be one way of bringing Cr(III) into contact with unevenly distributed Mn oxide surfaces (Bartlett, 1986). Mixing or churning of the soil would accelerate this process. Organic complexes may also inhibit Cr(III) oxidation by competing with Cr for Mn sites. Eh, pH and temperature, moisture and microbial populations will also all influence Cr oxidation in some fashion. All of the factors discussed will result in Cr(III) oxidation being less important in controlling Cr(III) levels in soil than precipitation/dissolution processes. This is illustrated by the heavier arrow to precipitation/dissolution from Cr(III) in Figure 1.

The Cr(VI) formed by oxidation of Cr(III) is also, in most soils, likely to be rapidly reduced again by any number of reductants such as ferrous iron, reduced sulphur species and organic complexes (Rai *et al.*, 1986; Bartlett, 1986; James and Bartlett, 1983b). Fe(II) containing minerals are common in soils and should provide sufficient Fe(II) in solution to reduce Cr(VI), except under extreme oxidizing conditions (Rai *et al.*, 1988). Cr(III) formed in this manner will then be controlled by the solubility of $Cr(OH)_3$ and/or $Cr_xFe_{1-x}(OH)_3$. Dissolved O_2, particularly in the presence of phosphates, will compete with Cr(VI) for Fe(II). Both the dissolved O_2 concentration and pH will determine the extent of competition by O_2 for Fe(II)(Rai *et al.*, 1988). Reduction of Cr(VI) to Cr(III) is marked C on Figure 1.

Cr(VI) in the form of chromate (CrO_4^{2-}) that escapes reduction might then be adsorbed on amorphous iron oxyhydroxides (Zachara *et al.*, 1987). CrO_4^{2-} adsorption is strongly influenced by pH, with acidic conditions resulting in greater adsorption. Behaviour of chromate in response to pH

was similar to that of phosphate (Rai *et al.*, 1988). Several common soil water constituents decrease chromate adsorption onto Fe oxides. Dissolved inorganic carbon species bind to oxide surfaces in a complex manner, resulting in the reduction of chromate adsorption by an electrostatic effect. (Zachara *et al.*, 1987). Silicic acid ($H_4SiO_{4(aq)}$) and SO_4^{2-} were also shown to depress chromate adsorption electrostatically. The effects of these compounds is additive, with the total suppression of adsorption varying from 50-80 % of that in a weak electrolyte (Zachara *et al.*, 1987). The presence of cations such as Ca, K and Mg has little counteractive effect. Thus, while chromate adsorption is an important mechanism in governing Cr(VI) levels, it is far more subject to environmental conditions than is the precipitation/dissolution of Cr(III). In those soils containing $BaSO_4$, a poorly soluble barium-chromium solid solution, $Ba(S,Cr)O_{4(ss)}$, will control the upper limits of Cr(VI) in solution (Rai *et al.*, 1988). These processes are marked as D and E respectively in Figure 1.

The chemistry of Cr in soils can be seen to be controlled by a variety of processes, depending on the presence of various oxides and solid solutions. The effectiveness of these processes in determining aqueous Cr(III) and Cr(VI) concentrations is modified by external environmental factors such as pH, Eh and the presence of a variety of ions and organic constituents (Figure 1). It is worth noting that earthworms, through their activities, could alter some of these modifying factors and thus influence the availability and mobility of Cr within the soil system.

2.1.3 Plants and Chromium

The interest in plants and Cr was originally sparked by the hope of increasing Cr(III) content in edible foods. People in high Cr regions have a lower mortality rate due to cardiovascular failure (Cannon and Hopps, as in Bartlett and Kimble, 1976). Plants accumulate Cr to varying degrees depending on the species (Adriano, 1986). Cr(VI) and Cr(III) are equally available to plants grown in Cr spiked nutrient solution (Huffman and Allaway, 1973; Breeze, 1973) In soil Cr(VI) appears to be more toxic to plants than Cr(III), with as little as 5ppm Cr(VI) killing tobacco plants in sandy soil (Adriano, 1986). Toxic action must occur in plant roots as extremely little Cr

is translocated from roots to shoots (James and Bartlett, 1984b; Keefer *et al.* 1986; Cary *et al.*, 1977). Shewry and Peterson (1974) found that less than 1 % of Cr taken up was found in shoots while Huffman and Allaway (1973) showed that less than 0.1 % of accumulated Cr was found in wheat or bean seeds. While Cr does not appear to be easily translocated in plants, it can interfere with the uptake of essential nutrients such as Ca, K, Mg and P (Turner and Rust, 1974). The plant itself can in turn affect the oxidation state of Cr. James and Bartlett (1984b) found that Cr(III) levels were higher in the rhizosphere than in surrounding soil or controls and suggested root exudates may have reduced Cr(VI) and contributed to the formation of soluble Cr(III)-organic complexes. Mechanisms of Cr uptake and toxicity remain unclear. Shewry and Peterson (1974) found most Cr in roots in a soluble non-particulate form in spatially isolated vacuoles. They proposed that Cr was taken up in a fashion analagous to Fe, as Cr(II). Other workers have suggested that Cr in plants interferes with Fe metabolism, resulting in Fe chlorosis (Grove and Ellis, 1980; Adriano, 1986). Thus while Cr does not appear to be bioaccumulated through plants, excess levels in soil will cause adverse effects. The use of Cr tolerant species in Cr(VI) contaminated soils might promote reduction to Cr(III) complexes.

2.1.4 Chromium and Microbial Populations

The influence of a heavy metal such as chromium on the soil microbial population is highly variable. The chemical nature of the element itself, the type and species of microorganism, and external environmental factors such as pH, clay and organic matter content, moisture and temperature all can affect the interaction between metal and microorganism.

Given the toxicity of some forms of Cr to fish and mammals, it is no surprise to find that amongst heavy metals examined, Cr is considered highly toxic to microorganisms. Chang and Broadbent (1982) and Beck (1981) found Cr the most inhibiting metal to microbial biomass transformations of six heavy metals studied. Juma and Tabatabai (1977) studied 20 trace elements and found that Cr(III) at 25 $\mu mol\ g^{-1}$ is a moderate to high inhibitor of both acid and alkaline phosphatase activity. As expected, the oxidation state of Cr is an important factor. Cr(VI) inhibits microbial growth much more than

does Cr(III) (Babich *et al.*, 1982; James and Bartlett, 1984a; Chang and Broadbent, 1982; Ross *et al.*, 1981).

The type and species of organism also influences the effects of the metal. Autotrophs are more resistant to Cr than heterotrophs (James and Bartlett, 1984a), fungi are much more resistant than bacteria (Zibilske and Wagner, 1982; Beck, 1981). Within the fungi some species are considerably more resistant to Cr. Babich *et al.* (1982) report that growth of one species was stimulated at Cr(VI) levels that severely inhibited other species. Ross *et al.* (1981) found that gram negative bacteria are far less resistant than gram positive bacteria. This difference in bacterial response may be due to differences in cell wall structure between gram negative and gram positive bacteria. Beveridge (1984) found that gram positive bacteria bind considerably more metal to cell walls than do gram negative bacteria, which he attributed to the fact that gram positive cell walls contain many layers of a peptidoglycan matrix while gram negative walls have only a monolayer. By binding more metal ions to the cell wall the gram positive bacteria may thus be able to prevent passage of that metal ion across the cell membrane. These differences in the ability of microorganisms to adapt to Cr, in conjunction with environmental conditions, may result in a shift in population structure, which in turn could affect nutrient cycling and decomposition. On the other hand, microorganisms, by altering the location and binding of metals such as Cr, could be useful in detoxifying a site enough to permit plant growth and inhabitation by soil invertebrates.

2.2 Earthworm Ecology

Earthworms have been observed by and of interest to naturalists and scientists since the late 1700's when Gilbert White in The Natural History of Selborne (1789) described earthworms as "the intestines of the earth" (Graff, 1983). This work and that of Charles Darwin, nearly a century later (1881), recognized the significance of earthworms in the functioning of the soil system. Over 50 years more was to pass before modern scientists again began to investigate the influence of earthworms on soil physical, chemical and biological processes (J.E. Satchell., M.B. Bouché and K.E. Lee for example). Earthworms are now cultured for bait (Tomlin, 1983; Altwin Distributors, personal communication) and animal protein (Guerrero, 1983), and used to alter the nature and toxicity of organic wastes (Mba, 1983; Hatanaka *et al.*, 1983; Hartenstein *et al.*, 1981a; Southwell and Maier, 1981). Earthworms are currently being used in the purification of household wastewater in rural Japan, by the NIIMI process (Nakamura, 1982).

Recently, earthworms have been considered as potential bioindicators of metal polluted soil (Helmke *et al.*, 1979; Martin & Coughtrey, 1982). Various workers have also considered the colonization (both natural and man assisted) by earthworms of reclaimed mine spoil sites (Rushton, 1986; Curry and Cotton, 1983; Pietz *et al.*, 1984). Despite this work and a few recent conferences concerning earthworms, they are still an under-studied order relative to both other invertebrates and higher order species, particularly given their significance (Bouché, 1985, ix).

2.2.1 Classification of Earthworms

Earthworms belong to the phylum Annelida, class Oligochaeta. They are coelomate, that is, the body is divided by septa into a series of segments and is filled with coelomic fluid. Their basic structure is that of a long, double cylinder, consisting of an outer wall of both circular and longitudinal muscles separated by the coelomic fluid from a tube like intestine also encased in circular and longitudinal muscles. Locomotion is achieved by waves of alternate contractions and extension of the opposing body muscles in conjunction with bristle-like setae which run down the length of the animal

(Lee, 1985). The anterior portion of the animal is extended while the rear setae keep the posterior portion fixed. Once maximum anterior extension is reached, the anterior setae are used to stabilize the animal while the posterior portion is retracted. This cycle is repeated continuously from segment to segment, allowing forward motion. Waves can be propagated in either direction, which permits both backward and forward movement (Lee, 1985). Burrows are formed either by inserting the narrowed anterior portions (prostomium) between soil particles and then expanding to force the soil aside or, if no space is available for insertion, by physical consumption of the soil. The type and extent of burrows formed depends on the species and its particular ecological niche. Differences between species can be used to place them within an ecological classification scheme.

Bouché (1977) developed a morpho-ecological classification for European Lumbricidae. Groups were distinguished by morphological characteristics which could be considered functionally significant. These groups are:

1) épigées: litter dwelling, highly mobile, do not make burrows, consume decomposing litter almost exclusively.
   e.g. *Dendrobaena tubida*
   Lumbricus cataneus

2) anéciques: live in burrows in topsoil layers but feed on dead vegetation from surface which is dragged into burrow for actual feeding. Burrows may be shallow or deep, usually permanent, castings at surface.
   e.g. *L. terrestris*
   Aporrectodea longa

3) endogées: subsurface dwelling, feed on organic rich portions of mineral soil; burrows are impermanent and continuously expanded, casts in burrows.
   e.g. *A. rosea*
   Octolasion cyaneum

This classification has been adopted by several workers (Scheu, 1987a,b; Nemeth & Herrera, 1982; Phillipson *et al.*, 1976). However, as Lee (1985) and

others point out, the ecological groupings of Bouché and also those of Perel' (1977) are based solely on observations and knowledge of western European Lumbricidae. These species amount to only 10 % of all earthworms and are therefore hardly likely to be representative, ecologically or otherwise. The soil and other environmental conditions of western Europe are different from those found in most of Canada; as such, perhaps these classifications do not apply well here.

Two terms that describe two major distinct groups are recognized worldwide. The term geophage refers to soil consuming species, while detritivore refers to litter consuming species. Obviously some species will fall in between, but in general, these are useful in distinguishing those species most susceptible to changes in surface litter or alternatively, to disturbance or contamination of the mineral soil. An example of a detritivore is the undescribed *Arctiostrotus* spp. which inhabits the thick litter layer (and also decaying logs) of soils on the west coast of Vancouver Island (Spiers *et al.*, 1986). The species used in this study, *Eisenia fetida*, is geophagous in that it consumes the substrate in which it lives; however, its preferred habitat is one rich in organic matter, such as manure or sewage sludge.

2.2.2 Basic Requirements of Earthworms.

The essential requirements for the continuing survival of earthworms as discussed by Lee (1985) are:

a) a food supply of adequate quality and quantity,
b) adequate oxygen supply,
c) protection from light,
d) adequate moisture regime,
e) temperatures suitable to the individual species,
f) pH range suitable to the individual species.

This is not an exclusive list. It assumes that given the above conditions, the environment that the earthworm functions in is neutral. This is probably true for most natural environments (excluding predation and disease) but is definitely not true for anthropomorphically altered environments. Soil contaminated with heavy metals is such an altered environment. The interactions of earthworms and heavy metals will be discussed in a

subsequent section. The remainder of this section will discuss the list mentioned above.

Food Supply

Earthworms are saprophagous, in that they consume organic detritus. The bulk of their food is likely to be dead vegetative matter; however, sufficient evidence exists to suggest that for some, if not most, earthworm species fungi, bacteria, protozoa and other microflora and fauna are an important part of the diet (Rouelle, 1983; Satchell, 1983; Hartenstein *et al.*,1981a, b; Neuhauser *et al.*, 1980). This is perhaps especially true for geophagous species, where the observed selection of organic rich material may in fact be selection for high microbial populations (Satchell, 1983). Shaw (1984) provides further support for the direct consumption and metabolization of microorganisms by earthworms.

Results of examinations of gut contents of earthworms have been used to determine whether a species is a geophage or a detritivore. These studies have clearly shown that for many species the nature of the diet depends in part on the geographic location, and thus, perhaps on availability of a given food source. Lee (1985) describes several studies that have reported that *Aporrectodea rosea* feeds on soil organic matter and is therefore geophagous, while another study found *A. rosea* to be an exclusive root feeder and hence a detritivore. *A. rosea* is a temperate region Lumbricid. Differences in diet become more pronounced when temperate and tropical earthworms are compared. Tropical grasslands are dominated almost exclusively by geophages while detritivores dominate in temperate zones (Lee, 1985).

Very little is known concerning the specific nutrient requirements of earthworms. Satchell (1967, as in Lee, 1985) suggests carbon is not limiting, given reported energy assimilation of no more than 15 %, but that nitrogen is limiting. Large amounts of both C and N are excreted by earthworms, particularly as compared to Ca or P. The effects of this excretion, and of changes in C and N cycling due to physical activities of earthworms have been examined in several studies (Scheu, 1987a,b; Spiers *et al.*, 1986; Shaw & Pawluk, 1986b; Mitchell *et al.*, 1982).

Oxygen and Light

Sufficient oxygen and protection from light are both necessary for earthworm survival. Earthworms exchange oxygen and carbon dioxide through the cuticle, which is underlaid by capillary blood vessels (Stephenson, 1930). The cuticle must be kept moist to permit gaseous exchange in solution (Lee, 1985). Factors affecting oxygen demand and respiratory ratio in earthworms include: body size, level of activity, oxygen tension, ambient temperature, and contact with the soil (Lee, 1985). In general, soil CO_2 concentrations do not appear to affect earthworm respiration, and short term exposure to anaerobic conditions is well tolerated. As with respiration, earthworm responses to light are also adaptions to inhabiting the soil. The "withdrawal reflex", a rapid contraction of the muscles and removal of the body from exposure, is the classic response of earthworms to high light intensity. Litter and surface dwelling species are more tolerant of low intensity exposure than are subsurface species (Lee, 1985).

Moisture

Given that earthworms are 65-75 % water (Oglesby, 1969, as in Lee, 1985, 33), must maintain a moist body surface for respiration, and excrete large quantities of urine, it is not surprising that they require moist soil conditions. Lee (1985) speculates that water losses of 10-20 % of body weight per day are common for earthworms and notes that in their respiratory and excretory systems earthworms are more similar to aquatic organisms than to other soil invertebrates. Earthworms are not physiologically well equipped to survive desiccation (Stephenson, 1930; Jamieson, 1981) although some species will retreat to greater depths and most will enter diapause if near surface moisture conditions are insufficient. Lee (1985) discusses the results of several studies indicating a preferred soil moisture content range of 12 to 30 % , and death occurring when 60-80 % of body water had been lost (50-70 % of total body weight). In general, earthworms function at moisture contents similar to those considered sufficient to maintain plant growth (pF 2.0-3.5) (Lee, 1985).

Temperature

As with moisture content, minimum and maximum lethal temperatures and optimal activity temperature ranges for earthworms are species and location specific. Species that are surface and litter dwellers generally tolerate both higher temperatures and more rapid fluctuations than do subsurface dwelling species. Earthworms respond to adverse temperatures by retreating to greater depths in the soil or by producing cocoons from which they will not emerge until conditions are once again suitable (Lee, 1985).

Geographical location also influences response to temperature. Tropical species have higher upper lethal temperature limits of 30-35 °C, while temperate species limits range from 25-30 °C (various sources summarized in Lee, 1985). In either case, the upper limits are low as compared to other organisms (Lee, 1985). This could be due to an inability to maintain adequate oxygen at high temperatures, given a Q_{10} of approximately 2 for earthworms (i.e. metabolic rate increases ~ 2 times for every increase of 10 °C in body temperature) (Lee, 1985, 40). Alternatively, Rigby (1968, as in Lee, 1985) showed that collagen fibres from earthworm body wall melt when in physiological saline solution at 34-40 °C. He suggested this might account for the lethal upper temperature limit of ~35 °C.

Reported optimum temperatures represent either field observations of temperatures at which maximum weight gain and reproduction occur or are based on laboratory studies of temperature preferences. In either case some species and geographic differences are apparent. European Lumbricidae generally have optima of 10-15 °C while tropical species have optima from 20-25 °C. A species such as *Eisenia fetida* while being a temperate species, has optima in the 18-25 °C range, reflecting its preference for manure or sludge heaps where fermentation may increase ambient temperatures.

Acidity

Again, as with both moisture content and temperature, optimal pH ranges are species and location specific. Shaw (1984) summarized the literature, noting that while most species inhabit soils with pH ranging from 4.0 to 7.5, there are recorded cases of earthworms inhabiting soils of both

higher and lower pH. Spiers *et al.* (1986) documented an *Arctiostrotus* spp. inhabiting soil with a pH of ~ 2.9. Emiliani *et al.* (1971, as in Lee, 1985) recorded a species inhabiting saline soils in Argentina with a pH of 9.4-9.8.

2.3 Earthworms and Heavy Metals

Heavy metals produced as waste and by-products of industrial processing, agriculture, and urban sewage are of considerable environmental concern. Much of this waste material is deposited on the soil, either as atmospheric fall out or directly as sludge or landfill. As consumers of soil and surface detritus earthworms are uniquely susceptible to exposure to terrestially deposited heavy metals. The effect of these metals on earthworm activity is of significance for several reasons. Areas that are heavily contaminated with metals may not support any population of earthworms, resulting in a decline in the mixing of litter and mineral soil (Scheu, 1987c) and a decrease in the rate of decomposition of that litter (Scheu, 1987b; Shaw and Pawluk, 1986; Spiers *et al.*, 1986; Mitchell *et al.* 1982; Reichle, 1977). If the earthworms do survive prolonged exposure to heavy metals, their numbers may be reduced and those surviving may be less active, which will also result in decreased influence on organic matter, decomposition, soil structure and water infiltration.

In addition to adversely influencing the soil environment, heavy metals accumulated by earthworms may be passed up the food web where they may become sufficiently concentrated as to be lethal. Predation of earthworms is common to many animals. Birds are the largest consumers of earthworms but foxes, shrews, hedgehogs, badgers and the like all have been observed to both forage for and consume significant quantities of earthworms (Macdonald, 1983). Toads, newts (Macdonald, 1983), and snakes (Reynolds, 1977) also may consume earthworms. Obviously, heavy metals absorbed and accumulated by earthworms could move up through the food chain in numerous ways. The potential environmental changes due to accumulation (lethal or otherwise) of heavy metals by earthworms render studies of the effects of metals on earthworms of considerable interest. Lee (1985) and Ireland (1983) have reviewed the interactions between earthworms and heavy metals.

2.3.1 Uptake and Excretion of Heavy Metals

The modes by which earthworms absorb and excrete heavy metals are of interest because they can potentially alter the subsequent fate of both the earthworm and the metal. Most research has concentrated on Pb and Cd, because of their extreme toxicity to humans and the large amounts produced industrially and, in the case of Pb, by automobile exhaust (Lee, 1985). Metals are generally assumed to be absorbed by earthworms across the intestine. McIntyre and Mills (1975, as in Lee, 1985) concluded that Pb can also be absorbed across the body wall. Fleming and Richards (1982) demonstrated that the mucoid coat surrounding *E. fetida* will bind and retain heavy metals, including Pb, and suggested that these metals are not passed across the cuticle itself. They suggested that the mucus may in fact act to prevent cuticular exposure to heavy metals and other adverse materials in the earthworm's environment.

If heavy metals found in earthworms are then assumed to originate from ingested material, it becomes obvious that the form and location of the metal in the soil becomes important. Litter feeding species are susceptible to airborne pollutants or materials applied to the vegetation itself. Ireland (1983) describes studies which demonstrated significantly lower earthworm populations in orchards treated with copper fungicides than in untreated orchards. Subsurface species, while not directly exposed to airborne pollutants, will consume more heavy metals found in the soil itself than will litter feeders. If the metal(s) are associated with organic matter, the likelihood of accumulation is higher. Beveridge (1984) showed that heavy metals are readily bound to bacterial cell walls, and that the binding sites subsequently act as nucleation sites for further metal accumulation. If, as suggested earlier, some earthworm species are preferentially consuming microorganisms they could also consume relatively large amounts of heavy metals.

The form of the metal is also important. Hartenstein *et al.* (1981b) showed that only 5 of 15 metals tested were toxic to *E. fetida* regardless of the form in which they were applied. Some of the remaining ten were toxic or inhibitory in some forms but not in others. Certain non-metal salts also exhibited this effect on *E. fetida*.. In an earlier study (Hartenstein *et al.*, 1980),

2500 ppm copper applied as $CuSO_4$ to sludge was lethal, while earthworms survived for several months in unamended sludge containing 1500 ppm Cu. Beyer and Cromartie (1987) found that the combination of species and metal form was important and commented that earthworms cannot successfully be used as biological indicators of pollution unless these relationships between species, soil and metal are more fully documented.

Excretion of the absorbed heavy metal depends in part on where the metal is deposited within the earthworm and also on species differences. Wielgus-Serafinska and Kawka (1976) considered the accumulation and localization of lead in *E. fetida*. They found Pb tissue levels and locations varied with the level of applied Pb, but found no direct correlation between soil Pb levels and tissue levels. They concluded that with increasing Pb levels, excretory mechanisms are stimulated. Andersen and Laurson (1982), after examining the bodily distribution of several metals, concluded that in *L. terrestris* lead is handled in at least three ways:

1) immobilization in the chloragocytes of the gut wall,
2) excretion through the calciferous glands,
3) storage and/or elimination through the formation of waste nodules (brown bodies).

Aporrectodea longa has poorly developed calciferous glands but deals with Pb more effectively than *L. terrestris* through more extensive use of waste nodules (Andersen and Laursen, 1982). This same study also clearly showed that different metals are handled differently by the same species. Lead is well known to interact with the calciferous glands, and to affect the concentration of Ca within the worm (Ireland, 1983; Ireland, 1979; Richards and Ireland, 1978). Earthworms grown in high Ca, high Pb soils accumulate less Pb than those grown in low Ca, high Pb soils (Andersen and Laursen, 1982; Ireland, 1979). This phenomena, in conjunction with other work, has been interpreted as indicating that Pb competes with Ca for sites within the calciferous glands. However, Pb accumulation has also been shown to result in loss of glycogen from the chloragocytes (Richards and Ireland, 1978), which are primary sites for glycogen storage (Jamieson, 1981). Jamieson (1981) discusses the function of chloragogen cells (or chloragocytes) and with Lee (1985) concludes that in addition to the storage of glycogen and waste products

such as ammonia and urea, chloragogen cells play an extremely important role in the accumulation and disposal of several heavy metals other than Pb. Both chloragogen cells and waste nodules can be sloughed off the intestinal wall into the coelemic fluid (Jamieson, 1981). They may then be disintegrated by phagocytic coelomocytes and the material subsequently excreted or accumulated in posterior coelemic sacs (Andersen and Laursen, 1982). Andersen and Laursen (1982) have outlined the possible disposal mechanisms for metal in *L. terrestris* (Figure 2). This diagram is most likely applicable to other species, with appropriate adjustments made in the importance of a given mechanism, such as the calciferous glands.

Once absorbed, metals will affect the functioning of the earthworm. Lee (1985) has thoroughly reviewed the literature concerning the accumulation and toxicity of heavy metals in earthworms up to 1983. Much of the work he discusses presents apparently contradictory results. For example, Lee (1985) presents the results of van Rhee (1975) who reported over 20 % mortality and severe inhibition of development to maturity for *Aporrectodea caliginosa* exposed to 1100 μg Zn g^{-1} soil. In contrast, Malecki *et al.* (1982) reported that 2000 μg Zn g^{-1} was required to produce any significant decrease in growth rates of *Eisenia fetida*. Consideration of more recent literature does not necessarily resolve the confusion. Pietz *et al.* (1984) found that Zn was accumulated least of six metals tested. They noted, however, that significant accumulation of Zn and Cd also occurred in control earthworms. Beyer and Cromartie (1987) and Carter *et al.* (1983) found similar results and suggested that earthworms may be able to regulate Zn uptake. They attributed this to the probability that Zn is essential for earthworms, as it is for most organisms.

Some general conclusions can be drawn from the literature regarding earthworms and heavy metals. Cadmium is considered to be the most toxic and readily accumulated metal of those usually examined (Pb, Cd, Zn, Cu and sometimes As, Se, Ni, Cr, Fe, Mn) (Beyer and Cromartie, 1987; Lee, 1985; Pietz *et al.*, 1984). Soil variables such as pH, organic matter, and P, K, Mg and Ca levels were thought, in addition to soil metal concentrations, to account for variance in observed earthworm metal concentrations (Ma *et al.*, 1983; Ma,

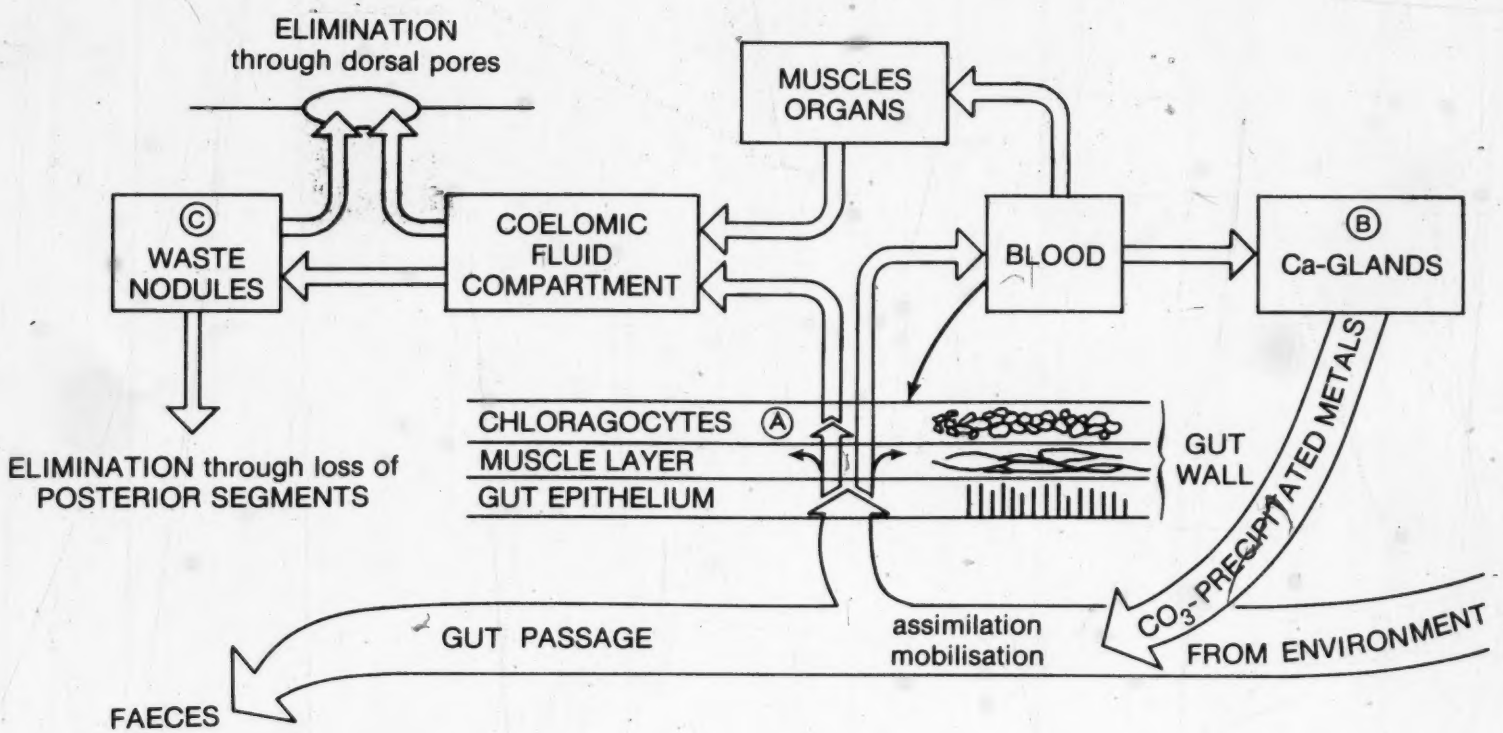


Figure 2. Metal flow through earthworm. A, B and C are three possible locations of metal accumulation (adapted from Andersen & Laursen, 1982)

1982; Andersen, 1979). Beyer *et al.* (1987) have shown that few if any of these variables have any significant correlation with earthworm concentrations of heavy metals. The exception was pH, which had a significant correlation with Pb levels and a probable effect on other metals. They concluded that for natural systems earthworm metal concentrations are directly related to soil metal concentrations but that this is not likely to be true for contaminated systems. In these systems, the form and location of the metal, and hence other soil variables, will be important in determining earthworm metal concentrations.

What the recent literature has emphasized is that results are probably specific to and must be explained in the context of the individual earthworm species studied, soil type and amendments used, and the source and form of the metal involved (Beyer and Cromartie, 1987; Pietz *et al.*, 1984; Ireland, 1983; Helmke *et al.*, 1979). Beyer and Cromartie (1987) have particularly emphasized this, in light of the growing use of earthworms as biological monitors of pollution (eg. Martin & Coughtrey, 1982).

Relatively little information exists regarding Cr and earthworms. Helmke *et al.* (1979) examined earthworms (*Aporrectodea tuberculata*) living in sludge amended agricultural soils for 28 elements, including Cr. They reported increased Cr in casts as sludge application rates increased but no corresponding increase in earthworm Cr levels, and concluded that Cr in sludge is biologically unavailable to earthworms. Hartenstein *et al.* (1981b) reported that 46,000 mg Cr kg^{-1} in sludge as Cr_2O_3 was innocuous to *E. fetida* grown in the sludge for 1 year. From these and other results, they concluded that the rich microbial population of the sludge somehow affected the toxicity of the metals to *E. fetida* and suggested results might be very different for contaminated mineral soils. Using several mineral soils of varying textures and 3 species of earthworm, Ma (1982) examined the uptake of several heavy metals, including Cr, by earthworms. Of the elements he considered, Cr was accumulated the least and appeared to be accumulated equally from all soils irrespective of texture or Cr soil level. No correlation was found between the cation exchange capacity (C.E.C.) and Cr, the only element where this relationship did not hold. He concluded that the low accumulation of Cr reflected its bioavalability relative to the other metals considered.

Pietz *et al.* (1984) also considered the effects of heavy metals on earthworms inhabiting sludge amended soils used to reclaim strip mine spoil. In sludge amended soils, decreasing metal accumulation was as follows: Cu > Cd > Ni > Cr > Pb > Zn. When concentration factors were compared for amended and non-amended, mined and non-mined soil, the ratios were not significantly different for Cr, Ni or Pb. Pietz *et al.* (1984) concluded from this that sludge applications had no effect on earthworm levels for these metals. These results conflict with those of Ma (1982) but are similar to those of Hartenstein *et al.* (1981). In addition to reporting Cr as least accumulated, Ma's (1982) results differ for other metals (Cd > Zn > Cu > Pb > Ni > Fe > Mn > Cr, as opposed to those of Peitz *et al.* (1984) above.). These differences might possibly be explained on the basis of the use of different earthworm species and mineral versus sludge amended soils in the two studies.

Beyer and Cromartie (1987) considered earthworm levels of Pb, Cu, Zn, Cd, Cr, As and Se for samples collected from 20 different sites. Metal concentrations in earthworms were poorly correlated with total soil levels (eg. r = -0.20 for Cr). They explained the poor correlations as a combination of three factors. These are: 1) the preference of earthworms for specific components of the soil, especially organic rich areas, 2) differences between species with respect to their ability to accumulate metals, and 3) the ability of earthworms to regulate both uptake and excretion of some essential metals, especially Cu, Zn, and Se. Examination of their Cr data illustrates their conclusions. Only one species, *Eisenoides loennbergi*, accumulated Cr to any great extent, and only at one site. That site did not have the highest soil Cr levels.

None of these studies was designed chiefly to look at the interactions of Cr and earthworms, and only one (Ma, 1982) appears to have deliberately chosen to add chromium to the soil rather than merely measuring it along with other metals that were considered to be of greater interest. In many respects this is not surprising. Until recently, few people appear to have recognized the ubiquitous use of Cr in industrial processes as a potential hazard. Little work was available concerning chromium-soil interactions, let

alone Cr-soil-earthworm interactions. Most Cr related research had focused on aquatic environments, where low levels of Cr(VI) are extremely toxic (NRCC,1976)

CHAPTER THREE: MATERIALS AND METHODS

In order to fulfill the objectives of the study, an artificial laboratory environment was created. This artificial system was necessary to control those variables which are known to affect earthworm activity, and to permit the even and accurate application of Cr to the soil. An artificial system, maintained in the laboratory, also permitted good control of the length and extent of exposure of the earthworms to Cr contaminated soil. Once more is known about the Cr-earthworm-soil system, field experiments might be appropriate.

3.1 Choice of Soil and Earthworm Species

The soil used in this study was Black Chernozemic Ah from a long term unseeded pasture in the Didsbury, Alberta area. The sod was stripped off and the top 5 cm of the Ah horizon removed. Soil was well mixed, air dried and ground to pass a 2 mm sieve. In preliminary studies this soil was used moistened to a predetermined moisture content. The Cr solutions however, lowered the pH of the soil from ~6.5 to pH 2-4. Earthworms cannot survive in soil of such low pH (Lee, 1985). Soil contaminated by industrial wastes is frequently very acidic. Earthworms could not be used to reclaim such soils nor could most plant species. Common reclamation practices include the liming of acidic soils and landfill. The soil for all subsequent experiments was therefore limed with 30 g $CaCO_3$ per kg soil. This rate was calculated to neutralize acidity produced by the addition of chromium, and was based on the amount of NaOH needed to titrate 50,000 pm stock Cr(VI) and Cr(III) solutions to neutrality. The resulting limed but non-Cr treated soil had a pH between 7.5 and 8. Once limed, the soil was placed in large pails which in turn were placed in open containers holding water (to approximately three cm depth) and allowed to equilibrate for several days. The pails were then removed, allowed to drain freely to slightly below field capacity and stored at ~15 °C until use.

Earthworm species fill a variety of ecological niches in the soil system (Reynolds, 1977) and react differently to the same stimuli (Lee, 1985). While recognizing this species diversity, it was felt that the purposes of this study

would be better served by using only one species, and thus avoiding possible interspecies effects.

Chromium was applied directly to the soil before the addition of the earthworms and would likely be adsorbed by amorphous Fe, Mn oxides (Rai *et al.*, 1986, 1988; Bartlett, 1986; Grove and Ellis, 1980) or by organic constituents (James and Bartlett, 1983b). Thus the species used needed to be at least partially, if not obligatorily, geophagous; that is, consumers of soil and associated organic matter for nourishment. Geophagous species are considered to belong to the éndogée ecological group (Bouché, 1977; Phillipson *et al.*, 1976; Lee, 1985). Chief characteristics of éndogée species include the construction of non-permanent burrows and the subsurface consumption of mineral soil and associated organic matter as a food source. These species are usually only active in the upper 10-20 cm of a soil. Species belonging to the anécique group construct permanent burrows and are often active at great depth (>1 m) in the soil (Bouché, 1977; Phillipson *et al.* 1976; Lee, 1985) Anéciques generally feed at the surface of the mineral soil or within the litter layer. Soil is only digested in large quantities when new burrows are created (Lee, 1985). *Lumbricus terrestris* is a common example of this group. Although found in Alberta over a wide range of soils, *L. terrestris* was not chosen for the study because of its feeding habits, which might preclude any significant consumption of soil applied metals. In addition, the use of earthworms for reclamation would generally require a species not dependent on the presence of a substantial surface vegetative layer.

The species *Eisenia fetida* was eventually chosen for use in the studies for the following reasons:

-it is a species commonly used in sewage sludge and industrial waste reclamation studies,

-it is geophagous, although if available, it prefers organic waste (This preference was not felt to be a problem since applied Cr is likely chelated by organic compounds in addition to being adsorbed onto portions of the mineral soil),

-it does not burrow deeply and therefore does not require extremely large containers.

-it is readily available in large numbers.

Upon arrival in the laboratory, earthworms were transplanted to pails containing the same 'Didsbury' soil used in all the experiments. The soil in the pails was moistened periodically and the pails covered with lids punctured by a sufficient number of fine holes to permit air exchange but not to allow earthworms to escape. The pails were stored in an incubator at a constant temperature of 12 ±2 °C.

3.2 Physical Set-up of Experiments

Both earthworm and non-earthworm incubation experimental units were housed in light and temperature controlled incubation chambers. Lighting was sufficient to approximate early spring daylight in Alberta (~10,000 foot candles), set for a 12-14 hour day. Temperature was also varied diurnally with a daytime maximum temperature of 18 ±2 °C. and a nighttime minimum of 12 ±2 °C.

Columns used in the study were made of cylinders either of clear plexiglass or grey PVC piping (inside diameter 8.1-8.3 cm) with a base of the same material. A 1 cm hole was drilled in the base to provide drainage and aeration. A thin disc of fine textured glass wool was placed in the bottom of the cylinder with a piece of fine plastic screening on top, to prevent loss of soil and/or earthworms from the base of the columns (Figure 3). Columns for earthworms were 20 cm in height and were loaded with 500 g of soil. Columns for non-worm portions of experiments were 10.5-11 cm in height and were loaded with 250 g of soil. In both cases more than enough soil was available for analysis. Five hundred g of soil was needed to provide sufficient depth and volume of soil to support five to seven earthworms (approximately 17 cm in depth and 898 cm^3. Clear columns were covered with black paper, which could be easily removed to permit observation of the columns. Plastic petri dish lids or bases were inverted over the column, preventing excessive moisture loss but permitting some gas exchange (Figure 3).

Chromium in solution was applied to the soil. The total solution applied moistened the soil to a moisture content corresponding to -1/2 bar, as

measured on a pressure plate apparatus. For the soils in this study this moisture content was ~ 42 %. A pressure of -1/2 bar was chosen as being close to, but not at, field capacity (-0.33 bar), thus providing a moist soil suitable for earthworms (Lee, 1985).

The Cr solutions were applied as a fine spray to a thin layer of soil spread out within a large clear plastic bag. Once all the solution had been sprayed onto the soil in an even fashion, the bag was shaken vigorously, to ensure as thorough mixing as possible. Sufficient solution and soil were used for any given application to ensure that all three repetitions of a particular treatment combination received an identical application of Cr. Consistent recovery rates of total Cr from non-worm columns indicate that this approach was successful (data not presented).

3.3 Outline of Experiments

3.3.1 Preliminary Experiment

Preliminary experiments were conducted to provide some estimates of useful working concentrations for both Cr(VI) and Cr(III), that is, levels at which earthworms are affected but not killed. The initial experiments used a single concentration of Cr(VI) and Cr(III), 5000 mg kg^{-1} soil. This level of application corresponds roughly to Cr levels commonly found in Cr contaminated industrial waste (Adriano, 1986) but is approximately 100 times greater than background total Cr levels for the prairies (McKeague and Wolynetz, 1980). The literature also suggested that earthworms could tolerate Cr concentrations far in excess of 5000 μg Cr g^{-1} soil (Hartenstein *et al.*, 1981b). Three replicates of the following treatments were prepared:

Treatment 1. no Cr, no earthworms,

Treatment 2. earthworms only,

Treatment 3. earthworms + Cr(III) as $CrCl_3$ applied at 5000 mg kg^{-1} soil,

Treatment 4. earthworms + Cr(VI) as CrO_3 applied at 5000 mg kg^{-1} soil.

500 g of the stored, partially moist soil was treated with 100 ml of H_2O containing sufficient Cr to produce an application rate of 5000 mg Cr kg^{-1} soil. Stock chromium solutions for use in these and subsequent applications were prepared as follows:

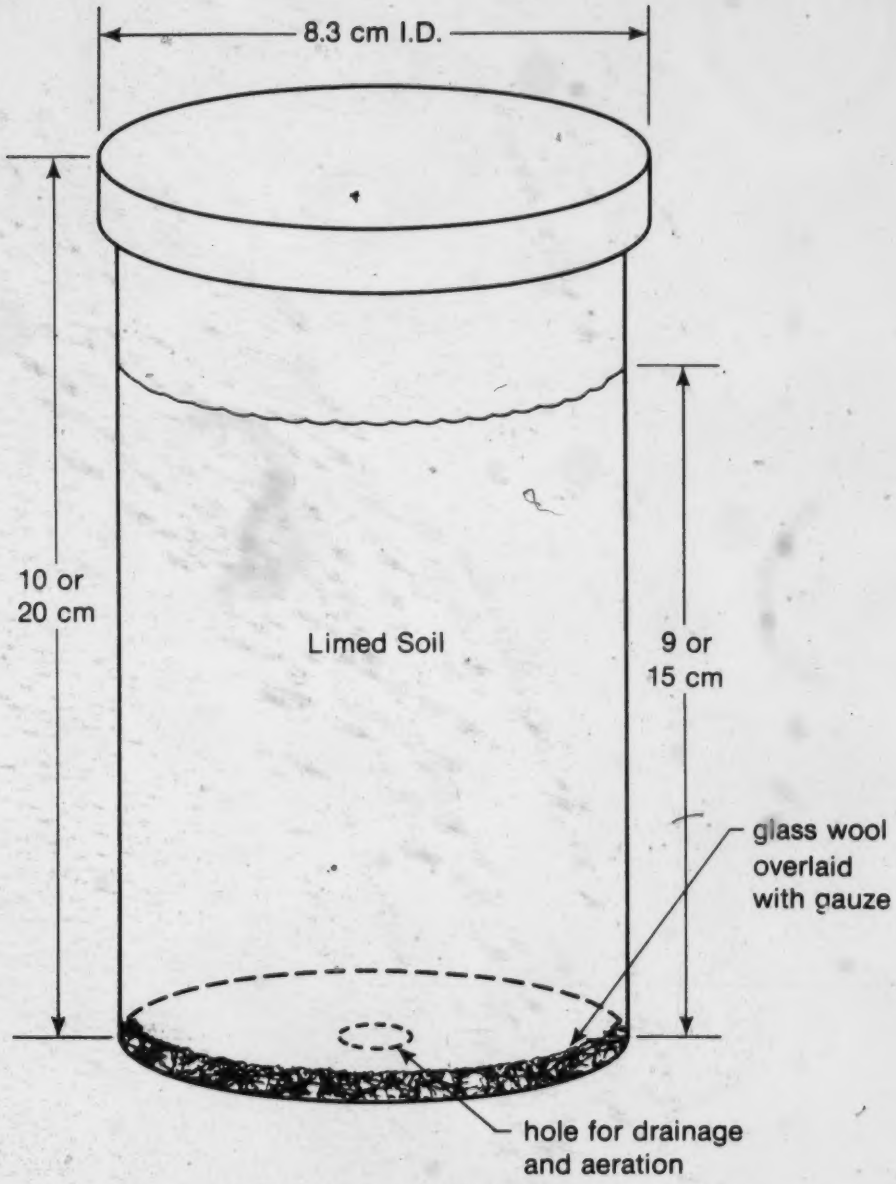


Figure 3. Schematic of plexiglas columns.

Cr(VI) as CrO_3: 15,000 ppm Cr(VI) stock prepared by dissolving 14.4232 g CrO_3 in 500 ml H_2O.

Cr(III) as $CrCl_3.6H_2O$: 50,000 ppm Cr(III) stock prepared by dissolving 128.065 g $CrCl_3$ in 500 ml H_20. Once the chromium applied soil was prepared, 600 g (initial soil weight +100 ml H_2O) was measured out and a portion sufficient to approximately half fill the column placed in a column previously prepared with glass wool and fibre mesh. Five (5) worms were then counted, cleaned of clinging soil particles by being lightly sprayed with water and added to the column along with the remaining soil. Burrows were not started in columns (mechanically), as the soil was loose enough to permit easy movement by the worms.

The worms in treatments 3 and 4 of this experiment all died within 36 hours of being loaded into the columns. Measurement of the soil pH indicated that the addition of the Cr solutions had lowered the pH from ~7.0 to 3.5. Low pH was considered to be a possible cause of death of the worms. The liming previously described was then undertaken in an attempt to provide a soil medium in which only Cr itself affected the earthworms. This limed soil was stored for a minimum of one month prior to use to permit reaction of the $CaCO_3$ with the Cr acidified soil.

3.3.2 Tolerance trial

Instead of merely repeating the above experiment with a limed soil, it was decided that a small Cr tolerance trial would be conducted. Accordingly, an experiment was conducted in which several application rates were evaluated. Three replicates of each of the following treatments were prepared:

Treatment 1: 100 mg kg^{-1} Cr(III)
Treatment 2: no chromium
Treatment 3: 500 mg kg^{-1} Cr(III)
Treatment 4: 1000 mg kg^{-1} Cr(III)
Treatment 5: 2000 mg kg^{-1} Cr(III)
Treatment 6: 3000 mg kg^{-1} Cr(III)
Treatment 7: 4000 mg kg^{-1} Cr(III)
Treatment 8: 5000 mg kg^{-1} Cr(III)

Treatment 9: 100 mg kg^{-1} Cr(VI)
Treatment 10: 500 mg kg^{-1} Cr(VI)
Treatment 11: 1000 mg kg^{-1} Cr(VI)
Treatment 12: 2000 mg kg^{-1} Cr(VI)
Treatment 13: 3000 mg kg^{-1} Cr(VI)
Treatment 14: 4000 mg kg^{-1} Cr(VI)
Treatment 15: 5000 mg kg^{-1} Cr(VI)

Each column within the earthworm treatment contained five worms. The columns were all to be incubated for 40 days and observed every 72 hours. The black paper covers were removed and the entire column was examined visually. If any worms within appeared to be dead, the column was removed from the incubator and sampled. Worms found dead at or near the surface were simply removed and tagged and the column left in the incubator. Some of the earthworm treated columns were pulled prior to 40 days because of the death of all of the worms in that column. Based in part of the results of this experiment subsequent experiments were designed to meet the other objectives of the study.

3.3.3 Earthworm - Cr Interaction Experiment

The initial experiments provided evidence that under certain conditions, Cr is toxic to earthworms. Preliminary results suggested Cr accumulation might be occurring. In order to meet the objectives of the study, further clarification was necessary. Using A) results from the tolerance trial, and B) known Cr distribution and forms under given pH and moisture conditions, one Cr application rate was chosen for each of Cr(VI) and Cr(III). Using 500 g columns containing seven (7) *E. fetida* individuals, the following experiment was conducted:

Three earthworm treatments:

(a) Earthworms added immediately after Cr application, E1,
(b) Earthworms added two days after Cr application, E2,
(c) No earthworms, NW.

Three Cr application rates:

(a) 0 mg kg^{-1} Cr,
(b) 75 mg Cr(VI) kg^{-1} soil, applied as CrO_3 solution,
(c) 1000 mg Cr(III) kg^{-1} soil, applied as $CrCl_3.6H_2O$.

Seven incubation dates: 1,2,4,8,16,32, and 64 days
3 replications of each treatment combination.

The number of earthworms per column was increased from five to seven in order to achieve sufficient dried sample weight for neutron activation analysis (INAA). The incubation dates were, for all intents and purposes, arbitrary. It was felt that most Cr-soil reactions would occur relatively rapidly, given the known kinetics of Cr adsorption/precipitation (Rai *et al.*, 1986,1988; Eary and Rai, 1987). Sixty four days was also seen as a long enough time period for earthworms to absorb and/or consume sufficient Cr to have an effect on their activity and hence on some related soil properties. Thus a geometric time series was seen as suitable for the incubation dates. Three replicates provided some measure of variability and enough degrees of freedom to conduct the necessary statistical analyses. The E2 treatment was conducted to determine whether exposure to Cr before it is 'completely' adsorbed/precipitated into the soil affects the toxicity of Cr to earthworms. A two day lag period was seen as sufficient since most Cr would likely have reacted in some way with the soil by that time.

3.3.4 Adsorption Experiment

Measurement of Cr adsorption and the subsequent application of one or more adsorption models might provide a reasonable estimate of the Cr application level beyond which added Cr is not adsorbed but remains in solution. This adsorption capacity may influence the toxicity of soil-applied Cr to earthworms. To measure the adsorption of Cr by the soil used in the study, the following experiment was conducted. The method used was a modification from Stollenwerk and Grove (1985): 20 ml of solutions containing 0, 25, 50, 100, 250, 500, 1000, 2000, 3000 or 4000 ppm Cr(VI) or Cr(III) were added to 4 ±0.05 g of limed soil in a 50 ml centrifuge tube. Three replicates of each treatment were prepared, resulting in a total of 3 reps X 10 application rates X 2 types of Cr = 60 tubes. The tubes were capped and shaken on a horizontal shaker for 5 days. The suspensions were then centrifuged at 16,000 rpm for 20 min. and filtered using a vacuum filter. The supernatant was retained and analyzed for Cr(VI) and total Cr.

3.4 Sampling Procedures and Laboratory measurements

3.4.1 Sampling Procedures

All columns were unloaded and sampled as follows. Once the columns were removed from the incubators, they were emptied into individual plastic pans and the earthworms sorted and separated from the soil. The number of live worms and the number missing (if any) was noted. Missing worms were assumed to have died and decomposed prior to the column being unloaded. Both live and dead worms were cleaned by repeated rinses with distilled water. Live worms were preserved using a modified version of a procedure described by Shaw (1984). This procedure results in straight, well preserved specimens that are suitable for identification. One earthworm from each treatment was preserved whole in 95 % ETOH and subsequently used for species identification confirmation. Freeze dried worms were crushed and weighed for total Cr analysis. Cr and Sc were measured in earthworm samples by instrumental neutron activation analysis (INAA). All INAA samples were weighed to 4 decimal places and sealed in plastic sample holders. Thirty four soil samples randomly chosen from among the treatments were also analyzed for Cr and Sc to provide an estimation of the recovery rate. Soil and vegetative reference materials (S0-4 #526 Chernozemic Ah and NBS Standard Reference Material 1573, tomato leaves, respectively) were used as checks on the accuracy and precision of the INAA data. Results for the reference materials fell within reported ranges for these materials (Bowman *et al.*, 1979; Koons and Helmke, 1978).

Scandium was measured in order to permit calculation of the amount of Cr remaining in the earthworms gut as Sc is inert and biologically unavailable to earthworms (Helmke *et al.*, 1979; Martin and Coughtrey, 1982). The soil was mixed to ensure homogeneity and then sampled and analyzed for:

(a) Moisture content: approximately 10 g weighed to 3 decimal places, in a foil tin, dried at 105 °C for 24 h and reweighed (McKeague, 1978).

(b) pH (both H_2O and 0.1M $CaCl_2$): approximately 10 g of soil and 20 ml solution, mixed, let stand for 30 min. and read on a calibrated meter.(McKeague, 1978)

(c) Solution chemistry: 5 ±0.05 g weighed and set aside for subsequent extraction.

(d) Soil Cr: approximately 1 g, weighed to 4 decimal places for analysis of total Cr by INAA (done on a representative subsample only).

(e) Biomass estimation: 50-75 g sieved through a 2 mm sieve and stored overnight at 15 °C prior to commencing biomass measurements. Any remaining soil was stored in a capped plastic container.

3.4.2 Biomass Estimation

Microbial biomass is commonly reported as a carbon to nitrogen ratio, based on measurements of biomass C and biomass N. Both biomass C and biomass N were measured in this study, using a modification of Jenkinson and Powlson's (1976) chloroform fumigation incubation technique (CFI). Biomass C was calculated from $[(CO_2\text{-C fumigated}) - (CO_2\text{-C unfumigated})]/k_c$ using a k_c of 0.411 (Voroney and Paul, 1984). CO_2 evolution was calculated from the volume of 0.5N HCl needed to bring the pH of the NaOH absorbing solution from ~10 to 3.80. Biomass N values were calculated from [(total N fumigated) - (total N unfumigated)]/k_n where total N, either fumigated or unfumigated, refers to (NH_4-N + NO_3-N), as measured in 2N KCl extracts of the soil used for biomass C measurements and k_n=0.68 (Shen *et al.*, 1984).

NH_4-N and NO_3-N were measured colorimetrically using Technicon Industrial Methods 98-70W and 487-77A respectively on a Technicon Autoanalyzer II. Three possible sources of interference were considered: a) the yellow colour of the Cr loaded samples might produce a response from the filters and detectors in the spectrophotometer, b) Cr(VI) might be reduced in the nitrate/nitrite reduction column and prevent its proper operation, and c) either Cr(VI) or Cr(III) might react with some of the reagents used in the procedure. These possible interferences were checked using two different tests. In the first test, samples with increasing amounts of Cr(VI) and/or Cr(III) were run through the autoanalyzer without any colour reagents added. Thus pure sample arrived in the spectrophotometer and it could be determined if the colour of the samples alone created a response. In the second test a series of NH_4-N and NO_3-N free standards was prepared that corresponded to the original application levels for the two Cr species, diluted

with 2M KCl and run as normal samples. This test thus checked for interference caused by the reaction of the Cr with the reagents. Neither test produced any evidence of interference, baselines remained constant throughout. It was concluded from these tests that neither Cr(III) nor Cr(VI) containing solutions will cause interference in either the NO_3^- or NH_4^+ colorimetric reactions. All results are means of single measurements made on each of the three replicates and are expressed on an oven-dry soil basis. Respiration, as $C0_2$-C evolved, was also calculated from this technique for all samples.

3.4.3 Solution Chemistry

Two sequential extractions were performed on the 5.0 ±0.05 g of soil set aside for solution chemistry. The first was an H_2O extraction designed to extract Cr already in solution in the soil. The second extraction was performed immediately following the first on the soil plug remaining after decanting the H_2O supernatant. The extractant used was 10mM KH_2PO_4/K_2HPO_4 (1:1 by weight). Both extractions followed the procedure described by Bartlett, (1986, also James and Bartlett, 1983c),using a 5:1 solution:soil ratio. The supernatant was filtered and the samples were then frozen until analyzed. Cr(VI) was measured in the samples within 48h of extraction by the s-diphenylcarbazide method (Reisenauer, 1982; Bartlett and James, 1979). The remaining extract was acidified to ~pH 1-2 using 10% HNO_3 and refrozen. Samples were later thawed and analyzed for total Cr(VI + III) by atomic absorption, using 2% NH_4Cl in the samples to prevent interference by soluble Fe (James and Bartlett, 1983c) and a N_2O-C_2H_2 flame to reduce general interference by a variety of other elements (Reisenauer, 1982).

3.5 Cr-Sc Ratio Calculations

Estimation of the amount of any given element in an earthworm is complicated by the fact that at the time of removal from the soil, the earthworms gut will probably contain appreciable and measurable amounts of the element. Various approaches have been taken to deal with this problem. The most common method is to remove the earthworm from the soil, wash it and place it in a clean petri dish containing filter paper for 4d (Hartenstein

et al., 1980; Ireland, 1979; Gish and Christensen, 1973).During this time the earthworm is starved and will egest all unabsorbable waste material from its intestinal tract. There is some evidence however, that starvation alters the amounts and locations of metal residues in tissues (Beyer, 1981; Richards and Ireland, 1978) and that soil starvation does not necessarily eliminate all soil residues from the earthworms gut (Helmke *et al.*, 1979). The physical removal of gut material prior to analysis also means that reported concentrations are lower than that to which a consumer of the earthworm might be exposed. The method described here for the calculation of the amount of Cr in earthworm due to material in the gut is a modification of the calculation of Helmke *et al.*, (1979). It makes use of the biological unavailability of Sc and, by not removing gut material, also permits estimation of the metal levels to which consumers further up the food-web might be exposed.

The significant difference in this correction from that described by Helmke *et al.* (1979) is that of assumptions A) and B) described below. In this study C^x_{gut} was assumed to be equal to C^x_{soil}. Helmke *et al.* (1979) assumed C^x_{gut} equal to C^x_{cast} but made no comment as to why C^x_{cast} was chosen. Given reported assimilation efficiencies of less than 2.5% for earthworms in temperate regions, C^x_{cast} for an inert element such as Sc should approximately equal C^x_{soil} (Lee, 1985; Bolton and Phillipson, 1976). Assuming $C^x_{gut} \approx C^x_{cast}$ for the element of interest is more tenuous however, as this requires assuming that all or most material in the gut at the time of death is already digested. Helmke *et al.* (1979) achieve this by starving the worms for 30h. However, as previously mentioned, starvation probably alters both the amount and location of the element of interest within the earthworm (Richards and Ireland, 1978; Beyer, 1981). Given these problems it was decided that assuming C^x_{gut} equal to C^x_{soil} was more appropriate. It should be noted that both approaches assume that the elements in question are evenly distributed throughout the soil, an assumption known to be false.

Essentially, the calculation involves the use of interelement ratios and a mass balance of each of the elements. The assumptions made are: A) that,

$$\frac{Cr_{gut}}{Cr_{soil}} \approx \frac{Sc_{gut}}{Sc_{soil}};$$

B) that C^x_{gut} is approximately equal to the concentration of the element in the soil, C^x_{soil}; and C) that no assimilation of the inert element occurs and therefore the measured concentration of that element in the worm actually is the concentration in the gut material, that is, the mass of element in worm divided by the mass of gut material (C^x_{gut}). In effect, B) assumes that the gut material at the moment of death is largely undigested and that the mass of nutrients removed from the gut material by the worm is small in comparison to the total mass of gut material. The former, assuming that gut material at the moment of death is largely undigested, is obviously an approximation, since some gut material will have been digested and material extracted and passed across the gut to other tissues. However, gut transit times for earthworms are short, relative to other organisms, and range from 0.85h to 2.5h for temperate subsurface species (Scheu,1987c; Hartenstein *et al.*, 1981b; Bolton and Phillipson, 1976). Therefore, the majority of material in the gut at any one moment will not have been passed through the small portion of the intestine where absorption occurs (Lee, 1985). The latter assumption, that the percentage of nutrients removed relative to the total gut material is small is also reasonable, in light of the small assimilation rates discussed previously.

Of the elements that have been shown to be inert and not accumulated by earthworms (Hf, Tb, Sc) (Helmke *et al.*, 1979), Sc was used in this study because INAA analytical procedures for this element are sensitive and precise (Helmke *et al.*, 1979; Koons and Helmke, 1978).

Beginning with Sc and assumptions B) and C):

$C^{Sc}_{gut} \approx C^{Sc}_{soil}$ can be written as,

$$\frac{\text{mass Sc worm}}{\text{mass of gut material}} \approx \frac{\text{mass Sc soil}}{\text{mass soil}} \qquad (1)$$

But the reported Sc ppm values for the worm are:

$$\frac{\text{mass Sc worm}}{\text{mass worm + mass gut material}} \qquad (2)$$

therefore,

$$\text{mass Sc worm} = (\text{reported Sc value})(\text{mass worm} + \text{mass gut material}) \quad (3)$$

Combining (1) and (3):

$$\text{mass gut material} = \frac{(\text{reported Sc value})(\text{mass worm} + \text{mass gut material})(\text{mass soil})}{\text{mass Sc soil}} \quad (4)$$

From assumption A) and applying assumption B) to Cr:

$$\frac{\text{mass Cr gut}}{\text{mass gut material}} = \frac{\text{mass Cr soil}}{\text{mass soil}} \quad (5)$$

$$\text{mass Cr gut} = \frac{(\text{mass Cr soil})(\text{mass gut material})}{\text{mass soil}}, \quad (6)$$

where (mass gut material) is from (4).

Having calculated the mass of Cr in the gut material, the actual correction to reported Cr values for worms:

$$\text{total mass Cr} = \text{reported Cr value (ppm)} * \text{weight total sample} \quad (7)$$

$$\text{mass Cr worm} = \text{total mass Cr} - \text{mass Cr gut} \qquad \text{(from (6))}$$

$$\text{corrected Cr worm (ppm)} = \frac{\text{mass Cr worm}}{(\text{weight total sample} - \text{mass gut material})} \quad (8)$$

This correction was applied to all INAA data for earthworm samples.

3.6 Data Analysis

Data analyses were performed by analysis of variance (ANOVA) procedures. Data were analyzed as a 3x3x7 factorial experiment with 3 types of Cr, 3 earthworm treatments and 7 incubation dates. Earthworm-loading rate data (tolerance trial) were not analyzed statistically. Comparisons between means were made using the protected least significant difference (PSD) procedure (Snedecor and Cochran, 1980). Under this procedure LSD's are only calculated where F is significant for the overall interaction.

CHAPTER FOUR: RESULTS AND DISCUSSION

This section presents and discusses the results of the experiments conducted as part of this study. All data pertinent to the initial Objectives (see Introduction) are discussed herein, in four parts. The first part presents the general chemistry and mineralogy of the 'Didsbury' soil used in this study and discusses the results of the Adsorption Experiment (see Materials and Methods 3.3.4). Part 2 deals with Objectives 1, 2 and 3 and discusses the extent and variation of Cr accumulation by *E. fetida*. Part 3 deals with Objectives 4 and 5 and discusses effects of Cr on *E. fetida* activity. Part 4 is not directly related to any one objective but relates attendant results concerning changes in Cr chemistry attributable to the activity of *E. fetida*. Differences between various treatment combinations, both in figures and as discussed, are statistically significant ($P < 0.05$) unless otherwise noted.

4.1 Chemistry, Mineralogy and Adsorption

Table 1 presents the chemistry and mineralogy of the 'Didsbury' soil. The Orthic Black Chernozem developed on till parent material. The pH and moisture content values are means over the entire study period as there were no significant changes in these parameters over the course of the incubation. The moisture content values are for the incubated soil and approximate the moisture content of the soil at ~ -1/2 bar pressure as measured on a pressure plate apparatus. Field capacity moisture contents would therefore be a few percent higher than values shown in Table 1. This soil has 11.3 % organic matter, which is consistent for soils of this type (Dudas and Pawluk, 1970). The clay suite is dominated by montmorillonitic clays, with hydrous mica, chlorite and kaolinite also present in decreasing amounts (Table 1). Both high amounts of organic matter and montmorillonite will have effects on the Cr chemistry. James and Bartlett (1983 a,b) suggested that organic constituents can increase soluble levels of Cr(VI) and Cr(III) and interfere in other soil-Cr reactions. Griffen *et al.* (1977) showed that montmorillonite adsorbed considerable amounts of Cr(III) and lesser amounts of Cr(VI). Thus, in this soil there appears to be sufficient clay and organic matter to significantly influence Cr chemistry.

TABLE 1: Chemistry and Mineralogy of the 'Didsbury' soil (Orthic Black Chernozem) used in this study.

Property Measured	Chromium treatments		
	no Cr	Cr(III)	Cr(VI)
moisture content(%)	47.1	45.4	46.2
pH H_2O[a]	8.3	8.0	8.2
pH $CaCl_2$[a]	8.0	7.9	7.9
O.M (%)	11.3	11.3	11.3
organic C (%)	6.57	6.57	6.57
total N (%)	0.51	0.51	0.51
NH_4-N ($\mu g\ g^{-1}$)	4.6	4.6	4.6
NO_3-N ($\mu g\ g^{-1}$)	1.3	1.3	1.3
Mg ($cmol(-)kg^{-1}$)	4.5	4.5	4.5
K ($cmol(-)kg^{-1}$)	1.09	1.09	1.09
Na ($cmol(-)kg^{-1}$)	0.7	0.7	0.7
Ca ($cmol(-)kg^{-1}$)	17.3	17.3	17.3
Ca (limed)[b]	53.3	53.3	53.3
montmorillonite[c] (%)	50+	50+	50+
hydrous mica[c](%)	23	23	23
chlorite[c] (%)	4 - 8	4 - 8	4 - 8
kaolinite[c] (%)	2 - 4	2 - 4	2 - 4
quartz[c] (%)	2 - 6	2 - 6	2 - 6
Fe_2O_3[c,d] (%)	0.10	0.10	0.10
Al_2O_3[c,d] (%)	0.08	0.08	0.08

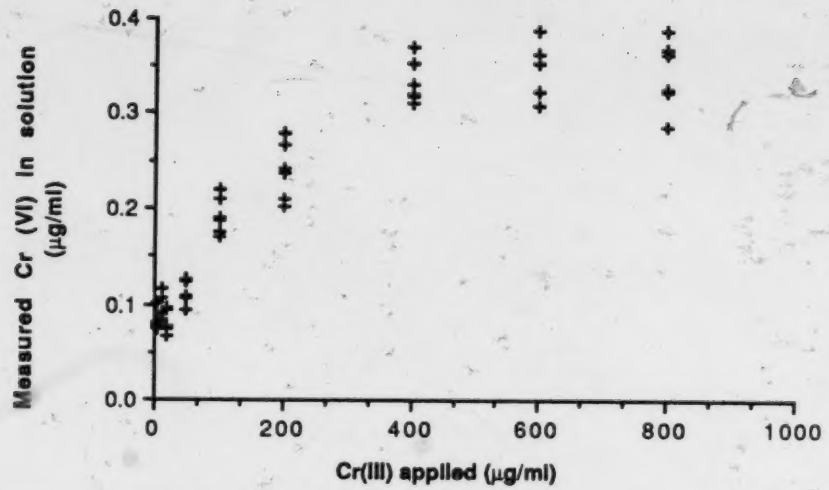
a. pH of limed soil, 0.01M CaCl2.b. Limed solution Ca calculated from addition of 30.0 g CaCO3 kg-1plus original Ca in soil and then subtracting the amount of Ca assumed to be held in the exchange complex.c. Data from Dudas and Pawluk, 1970.d. Acid ammonium oxalate extraction.

Figure 4 illustrates the results of the adsorption experiment. None of the isotherm equations commonly used to describe adsorption behaviour adequately described the data (figures not presented). What is shown in Figures 4a and b is the measured solution Cr(VI) versus the chromium applied, either Cr(III) or Cr(VI). "Total" Cr levels (Cr(III) + Cr(VI)) were below detection limits for the Cr(III) treatment and were almost entirely composed of Cr(VI) in the Cr(VI) treatment; they are therefore not presented for either treatment.

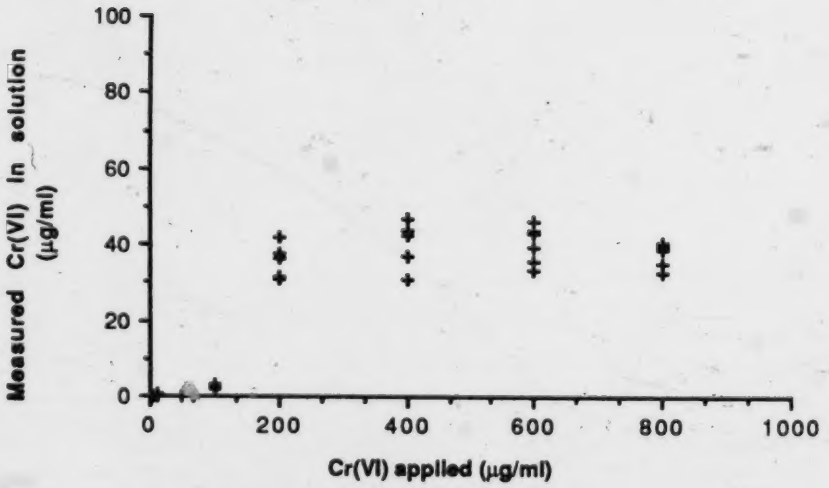
Solution levels of Cr(VI) in the Cr(III) treatment (Figure 4a) increase with increased application levels but level off at approximately 400 $\mu g\ ml^{-1}$ applied Cr(III). These "maximum" solution levels are extremely low, in all cases below 0.40 $\mu g\ ml^{-1}$ (7.7×10^{-6} mol Cr L^{-1}), in keeping with the low solubility of Cr(III) demonstrated by Rai *et al.* (1987) (10^{-6} to 10^{-7} mol Cr L^{-1} over pH 6 - 11). Precipitation/dissolution of $Cr(OH)^3_{(ss)}$ controls the solution levels of Cr rather than adsorption/desorption reactions, as indicated earlier in Figure 1. The pattern exhibited by the Cr(VI) treatment (Figure 4b) is somewhat different. Solution Cr(VI) levels are low until the 200 μg Cr(VI) ml^{-1} application level, whereupon they jump to 35 - 45 μg Cr(VI) ml^{-1} and remain so at all subsequent higher applications. This suggests that the soil reaches a maximum capacity to sorb, reduce or otherwise remove Cr(VI) from solution somewhere between 100 and 200 $\mu g\ ml^{-1}$ applied Cr(VI). The difference in solution Cr(VI) levels between the Cr(III) and Cr(VI) treatments illustrates that a soil's Cr sorption capacity is highly dependant on the oxidation state of the applied Cr. It is worth noting that solution Cr(VI) levels in the Cr(III) treatment constitute the majority of the Cr measured, indicating that Cr(III) not sorbed by the soil is oxidized to Cr(VI). These adsorption experiment results confirm that the reaction processes and mechanisms between Cr and soil constituents demonstrated theoretically and in pure systems by Rai *et al.* (1988, 1987) likely also apply to the soil used in this study.

4.2 Accumulation of Cr in *Eisenia fetida*

Other workers, from previous studies, have suggested that *E. fetida* does not accumulate Cr (Beyer and Cromartie, 1987; Hartenstein *et al.*, 1980; Helmke *et al.*, 1979) and that no adverse effects occur even when earthworms



A. Cr(III) treatment



B. Cr(VI) treatment

Figure 4: Measured Cr(VI) in solution*versus* A. Applied Cr(III), and B. Applied Cr(VI)

are exposed to extremely high levels of chromium (46,000 mg kg^{-1} in one study, Hartenstein *et al.*, 1981). The findings of this study directly contradict these previous results.

Accumulation, as a term, needs to be precisely defined to distinguish it from what is often called 'concentration'. Concentration, as defined by Van Hook (1974), is said to occur when the ratio of the concentration of the element in the organism (or tissue) to the concentration of the element in the environment is greater than one. That is,

$$\text{Concentration factor, (CF)} = \frac{[M]_{\text{organism}}}{[M]_{\text{envt.}}} > 1.0$$

Alternatively, accumulation can be defined as occurring when the concentration of an element in an organism or tissue increases relative to control individuals, as a result of exposure to that element (Hartenstein *et al.*, 1980). This type of accumulation is generally measured over an extended time of exposure. While CF ratios are commonly used (Beyer and Cromartie, 1987; Lee, 1985; Martin and Coughtrey, 1982; Ireland, 1975; Van Hook, 1974) they can be misleading. A CF ratio could be greater than 1.0 at a given application rate and less than one at a higher rate, due simply to physicochemical limits on the organisms' ability to take up the element. The organism may indeed have taken up more of the element in question at the higher application rate than at the lower rate but not be classified as having done so simply because body concentrations do not exceed high ambient levels. Therefore, for the purposes of this study, a distinction is made between concentration (CF ratios > 1.0) and accumulation (increased levels over time relative to controls).

Concentration of Cr by *E. fetida* occurred only for the Cr(III) treatment, for both the E1 (earthworms added immediately after Cr) and E2 (earthworms added after two days) treatments (Figures 5 and 6 respectively). CF ratios for Cr(III) increase over time, from approximately 1.0 after a one day incubation to 13.5 after 32 d for E1 and 16.5 after 64 d for E2. CF ratios for Cr(VI) and for the no-Cr control are less than one for both E1 and E2. While the difference in Cr uptake between Cr(III) and either Cr(VI) or no-Cr is obvious from

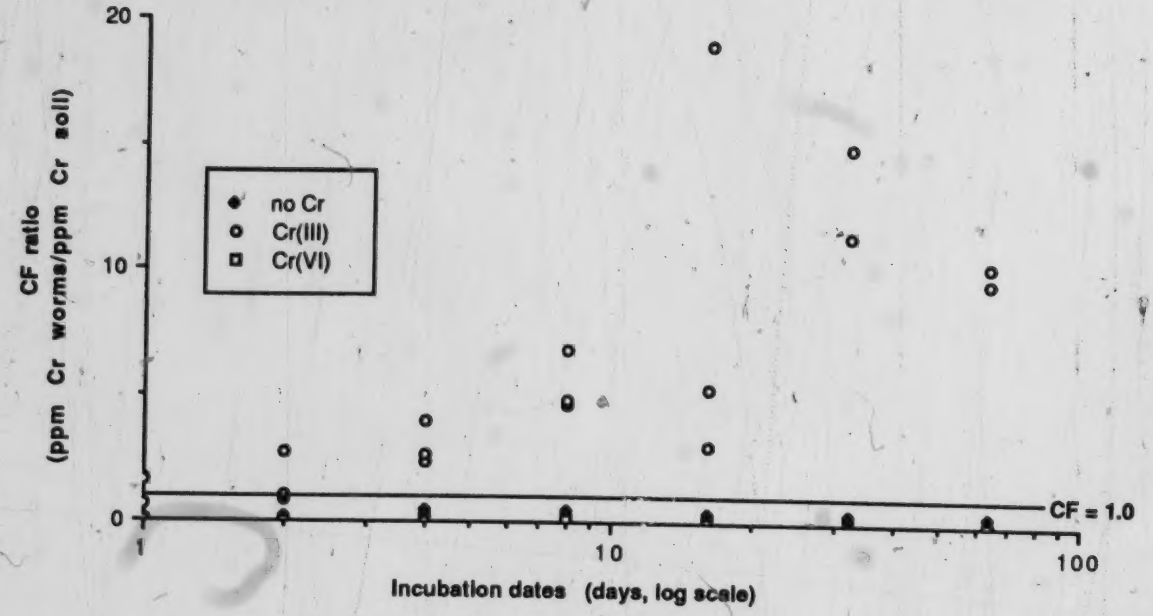


Figure 5: CF ratios for E1 treatment

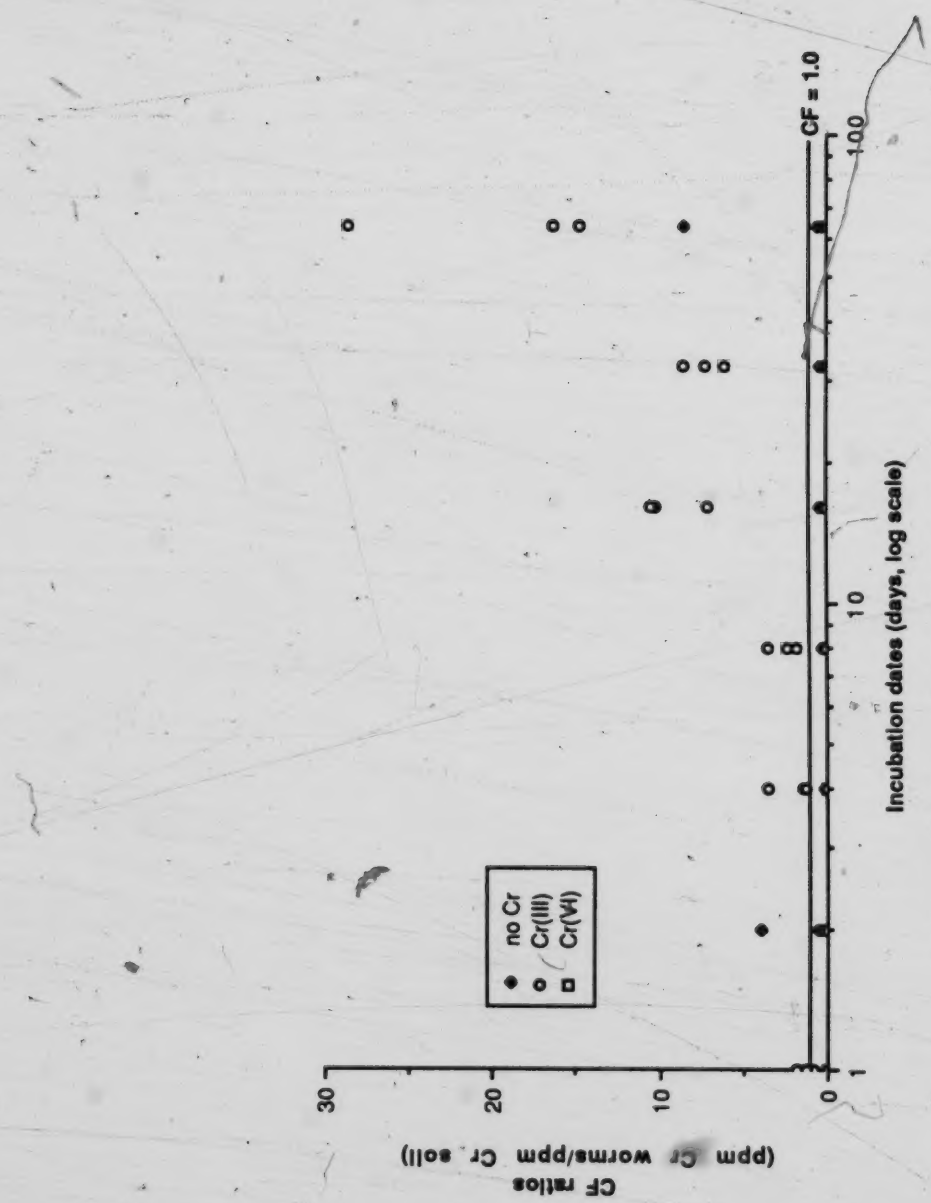


Figure 6: CF ratios for E2 treatment

Figures 5 and 6, possible differences between Cr(VI) uptake and the control cannot be discerned. The fact that the CF ratios for Cr(VI) and no-Cr appear so similar does not mean that the earthworms from each treatment contained similar amounts of Cr. Rather, this illustrates well the inherent weak$_n$ess in CF ratios.

Figure 7 compares Cr accumulation (not concentration) in the Cr(VI) treatment to the control, for the E1 treatments. From this, it can be clearly seen that accumulation of Cr occurred during the 4 and 8 d incubations. Accumulation possibly also occurred at later dates but low final earthworm sample weights (due to the death of the worms prior to the end of the incubation) resulted in either no data or values below the detection limit. No Cr accumulation occurred for the E2-Cr(VI) treatment (Figure 8). Cr accumulation for both E1-Cr(VI) and E2-Cr(VI) was extremely low compared to E1-Cr(III) and E2-Cr(III) accumulation (Figures 9 and 10). For both E1-Cr(III) and E2-Cr(III) treatments, Cr was accumulated by *E. fetida* and this accumulation increased over time.

With the exception of the E2-Cr(III) 64 d value, Cr accumulation was greater over all dates for E1 than for E2. This suggests that the two day lag between Cr application and earthworm introduction allowed sufficient time for some of the applied Cr(III) to become biologically unavailable. Rai *et al.* (1986) demonstrated that Cr concentrations in solution are controlled by $Cr(OH)^3_{(ss)}$ and, under acidic conditions and relatively low mole fractions of Cr, by $(Fe, Cr)(OH)^3_{(ss)}$. Both of these solid solutions exhibit rapid precipitation/dissolution kinetics (5 d or less) and solubility between pH 6 - 10.5 is below the drinking water standards established by the U.S. Environmental Protection Agency (Rai *et al.*, 1986). Given that the pH range of the amended soil used in this study (pH 7.9 - 8.3) is well within the range noted above, it is reasonably certain that Cr(III) added to amended soil would have precipitated as either $Cr(OH)^3_{(ss)}$ or $(Fe, Cr)(OH)^3_{(ss)}$. This is confirmed by the extremely low solution Cr levels seen for Cr(III) treated soil in the adsorption (Figure 4). Differences in uptake of Cr by E1-Cr(III) and E2-Cr(III) earthworms, might therefore be due to a change in the bioavailability subsequent to precipitation.

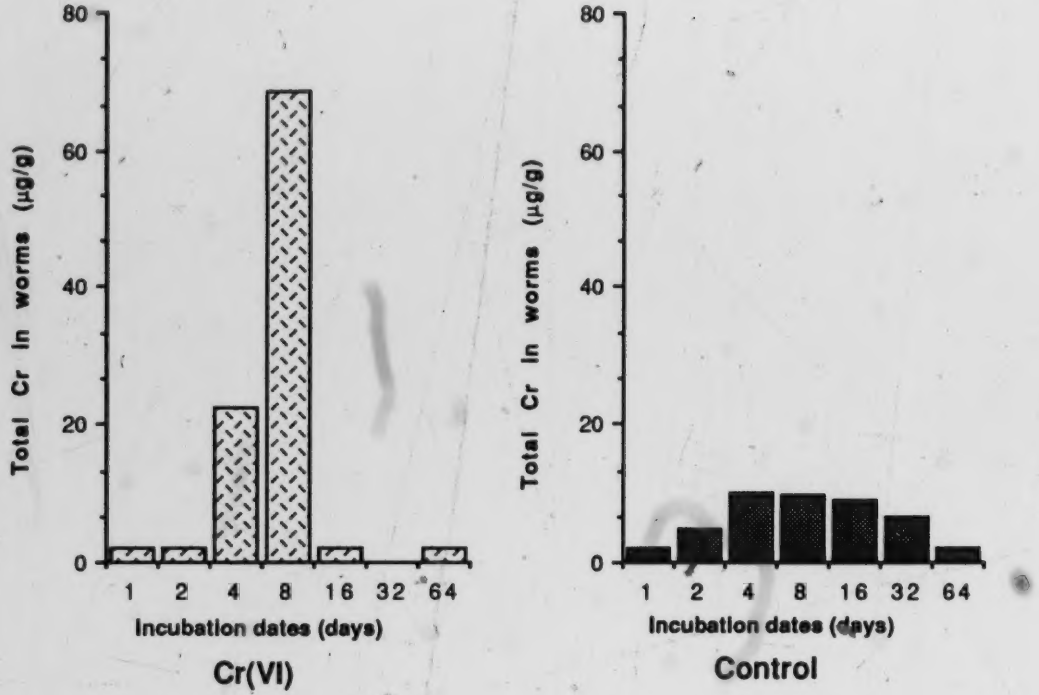


Figure 7: Total Cr in worms vs. Incubation for Cr(VI) and control, E1 treatment

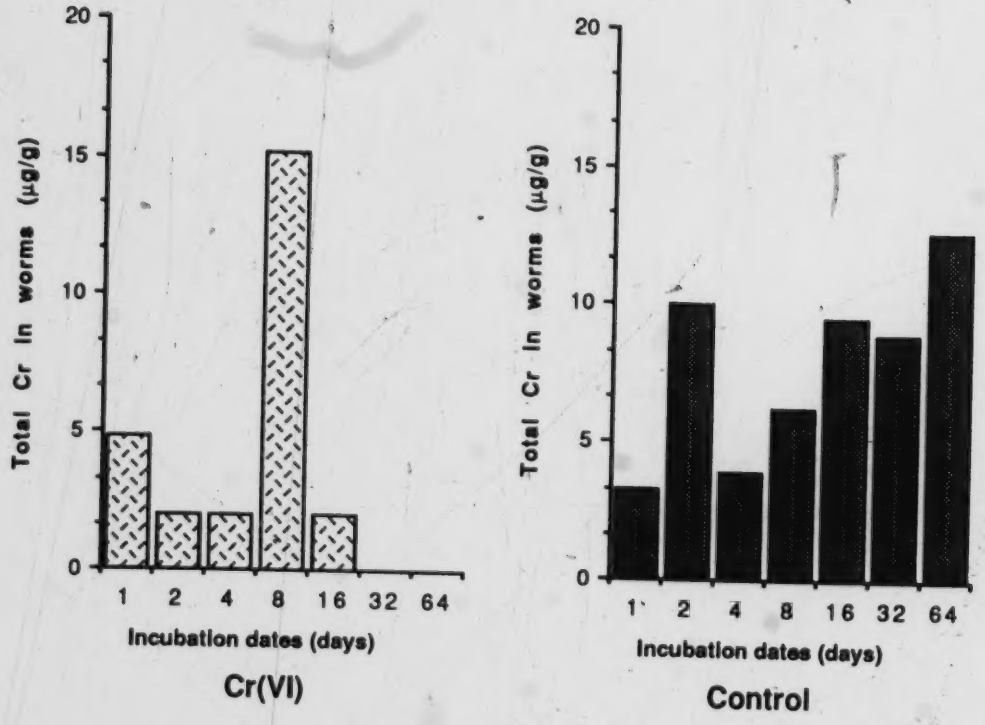


Figure 8: Total Cr in worms vs. Incubation for Cr(VI) and control, E2 treatment

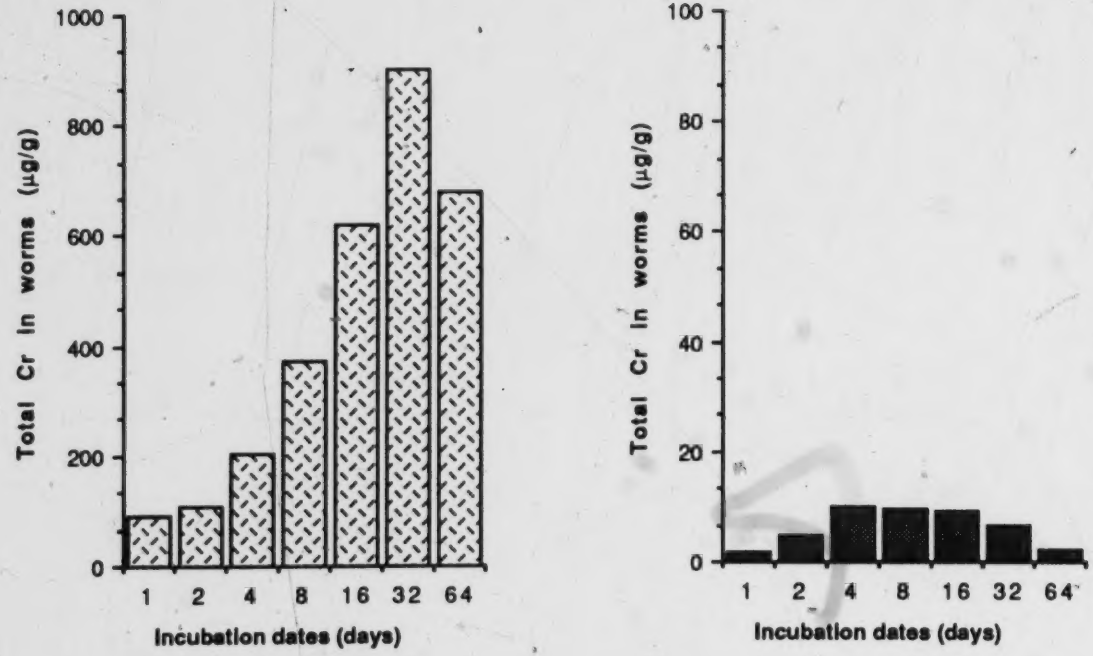


Figure 9: Total Cr in worms vs. Incubation for Cr(III) and control, E1 treatment

(Note: Cr(III) scale is ten times control scale)

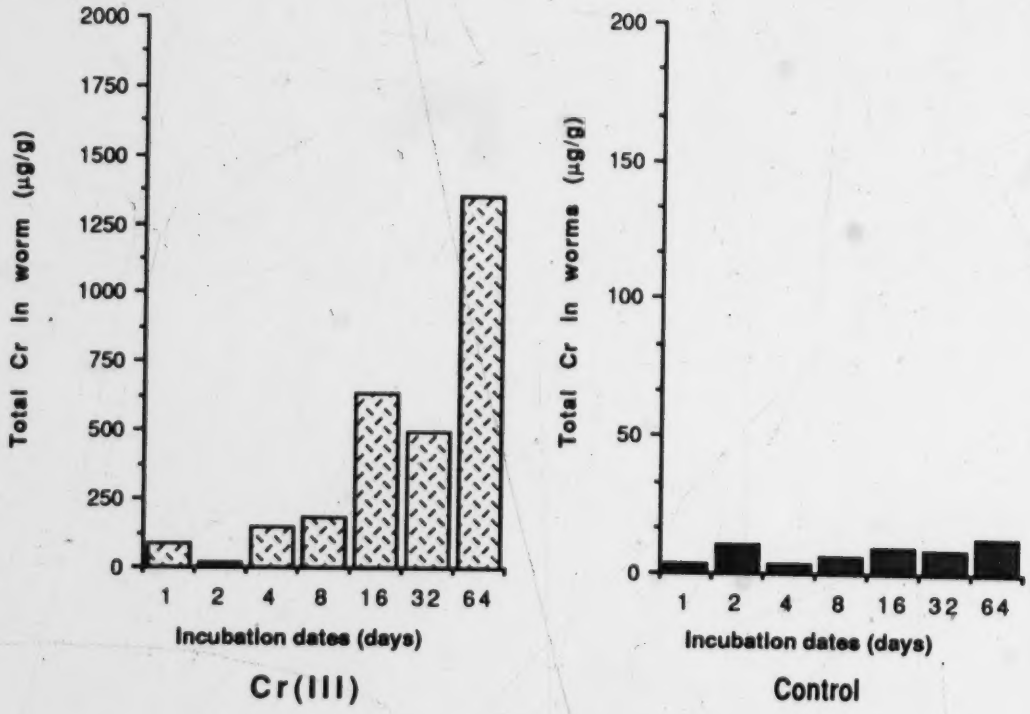


Figure 10: Total Cr in worms vs. Incubation for Cr(III) and control, E2 treatment

(Note: Cr(III) scale is ten times control scale)

Figure 11 shows the total Cr in worms versus the soil applied Cr for both the Cr(III) and Cr(VI) treatments. For Cr(III), no change in the amount of Cr accumulated occurs, regardless of the application level. The amount of Cr(III) accumulated is low, especially when compared to levels shown in Figures 9 and 10. In contrast, increasing amounts of Cr(VI) are accumulated as the level of Cr(VI) application increases. These confusing results can be clarified somewhat by considering the level of Cr loading in relation to a different earthworm measure, the percent kill (%kill). Percent kill was calculated as the number of dead *E. fetida* individuals divided by the total number *E. fetida* individuals originally added to the column. When %kill is plotted against Cr loading (Figure 12) it can be seen that the Cr(VI) treatment affected *E. fetida* much more than did the Cr(III) treatment, with 100 %kill reached at the 500 ppm Cr(VI) application, for an incubation of 2 - 5 days. From Figures 11 and 12 it can be concluded that while *E. fetida* accumulates greater Cr with increased application of Cr(VI), the Cr(VI) is almost certainly toxic at levels greater than 100 ppm. On the other hand Cr(III) does not appear to accumulate in increasing amounts as the level of application is increased, although the higher levels are more toxic. Cr(III) is, however, definitely accumulated over time (see Figures 9 and 10).

Having shown that Cr is accumulated by *E. fetida*, the relationship between % kill and various Cr fractions was examined. The Cr fractions considered were:

H_2O extractable Cr (III + VI),
H_2O extractable Cr(VI),
K_2HPO_4 extractable Cr (III + VI),
K_2HPO_4 extractable Cr(VI),
total soil Cr (III + VI),
non-recoverable Cr.

The H_2O extraction represents Cr in solution and the K_2HPO_4 extraction represents loosely bound Cr. Non-recoverable Cr (NRCr) was calculated as total soil Cr - (H_2O extractable + K_2HPO_4 extractable Cr) and represents Cr that is moderately to strongly bound by the soil. No correlation was found to exist between %kill and any of the four extractions, or total soil Cr. The relationship between %kill and NRCr varied with the type of Cr applied. For Cr(III), $R^2 = 0.20$, while for Cr(VI) $R^2 = 0.67$ ($P < 0.05$ in both cases). From these

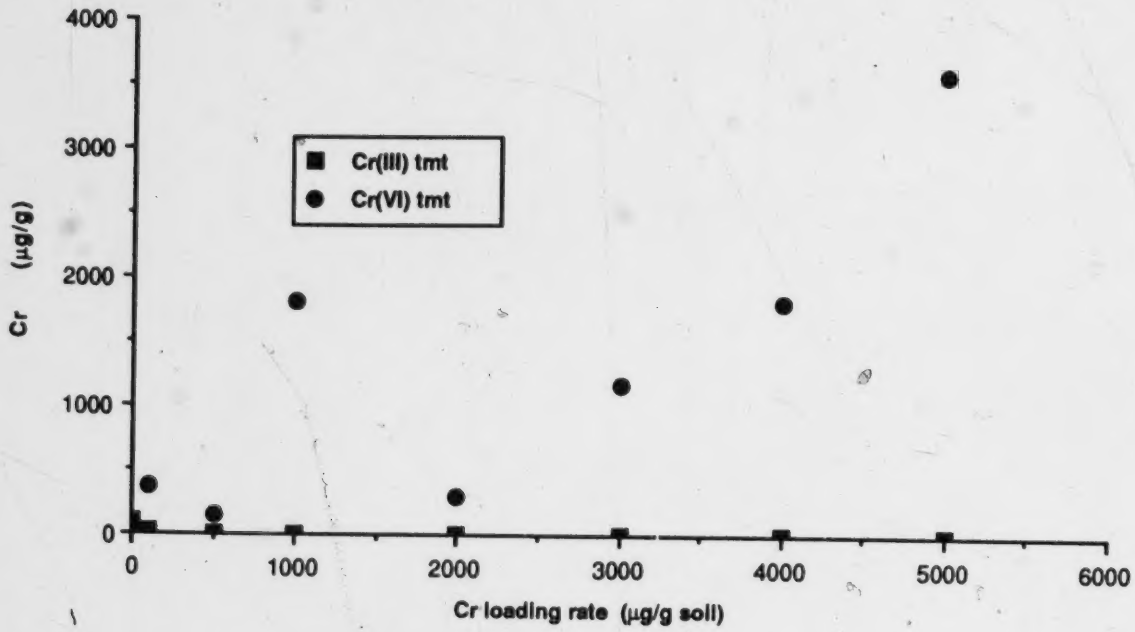


Figure 11: Total Cr in worms vs. Soil applied Cr

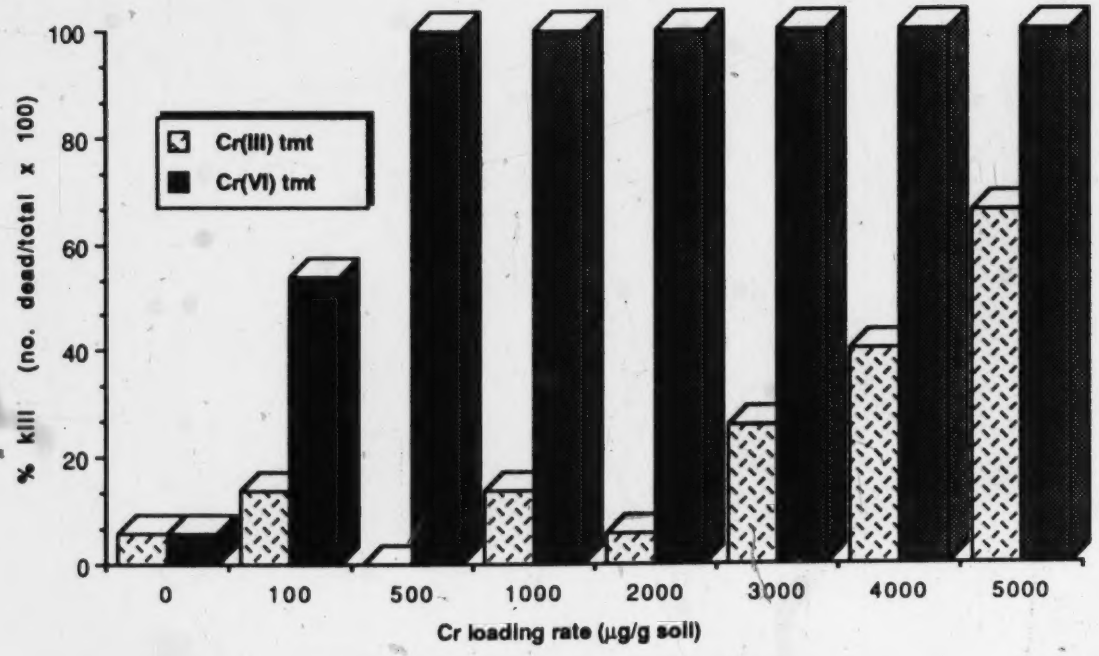


Figure 12: "Percent Kill" vs. Soil applied Cr

results it is not possible to unequivocally state that non-recoverable Cr is directly affecting mortality of *E. fetida*. The results do suggest that for applied Cr(VI), the amount of bound Cr does influence earthworm mortality, but the effect may be indirect. Cr(VI) is generally less tightly bound in soils than Cr(III) (Rai *et al.*, 1988). Fluctuations in NRCr in Cr(VI) soils over time are therefore more likely to occur and indirectly affect the amount of Cr to which an earthworm might be exposed.

The combination of increasing Cr accumulation with increasing levels of Cr(VI) application (Figure 11) and 100 %kill for ≥ 500 ppm Cr(VI) over a short time period (Figure 12), taken with the unclear Cr accumulation over time when Cr(VI) was applied at 75 ppm (Figures 7 and 8), suggests that Cr(VI) is easily taken up and retained by *E. fetida*. Cr(III) appears not to be as easily taken up but has less toxic effects than Cr(VI) once retained. These observations are consistent with the behaviour of Cr in other organisms. As an uncomplexed cation Cr(III) cannot be readily absorbed across the gut wall nor pass cell membranes. This lack of mobility and its low concentration in natural systems would appear to render Cr(III) of less toxicological importance than Cr(VI) (Jaworski, 1984). Cr(III) is a necessary mammalian micronutrient. The biologically active species is a dinicotinic acid - glutathione - Cr(III) complex called the glucose tolerance factor (GTF) which is readily absorbed across the gut wall (Jaworski, 1984; Mertz, 1979; NRCC, 1976; Mertz, 1969).

Cr(VI) ions are generally anionic and small in size relative to complexed and uncomplexed Cr(III) ions (NRCC, 1976). Both of these characteristics permit the passage of Cr(VI) across cell membranes and intestinal wall (Mertz, 1979; NRCC, 1976). Once inside a cell Cr(VI) acts as an enzyme poison. It is likely reduced to Cr(III) and 'poisons' the enzyme by stabilizing it in an incorrect configuration (NRCC, 1976). Cr(VI) reduced to Cr(III) may also complex with ATP and then compete with Mg-ATP as RNA and DNA substrate. Both of these processes are irreversible.

While the research in the preceding paragraphs was conducted on organisms other than earthworms, there is no reason to assume that the in-cell biochemistry of Cr(VI) is markedly different in earthworms. Sediment dwelling marine polycheates were shown to have a threshold toxicity of 1

ppm for Cr(VI)(Raymont and Shields, 1963). From this discussion and the results illustrated in Figures 7 through 12, it is apparent that Cr(VI) is more readily taken up by earthworms than is Cr(III) and once retained is considerably more toxic.

These differences between Cr(VI) and Cr(III) uptake and behaviour have implications for Cr movement through food webs. Cr(VI), at common levels for industrial waste (100 - 2900 ppm, see Adriano, 1986; NRCC, 1976), would be toxic to exposed *E. fetida* and probably also to other species, regardless of how much was actually accumulated. This is well illustrated for *E. fetida* in Figures 11 and 12. The earthworm would then be decomposed and the Cr would remain in the soil system. In contrast, Cr(III) levels in soil over most of the industrial range given are not toxic (see Figures 11 and 12). *E. fetida* and presumably other earthworms could conceivably live long enough to accumulate fairly substantial amounts of Cr(III) (Figures 9 and 10) and then be consumed.

The organisms that consume the earthworms will also consume any Cr in the gut material. Of the total Cr measured (Cr in worm + Cr in gut) an average of 47.4 % was in the gut material, with a range across all treatments from 4.4 to 99.6 %. This wide range is probably a result of several factors: 1) the spatial variability in soil of both the Cr and inert Sc used as a correction factor - a variability not accounted for by the mass balance correction technique, 2) the variation in feeding patterns between individual earthworms, and 3) variation in absorption of Cr by individual earthworms due to the presence of varying types and amounts of mineral and organic material in the gut. The reasonable standard errors in worm Cr amounts within individual treatment combinations, and the correlation of high gut Cr percentages with the higher levels of Cr application, suggest that the variation is not sufficient to significantly alter the trends seen in this study (data not presented). Nonetheless these results do indicate that earthworm consumers could be exposed to greatly increased amounts of Cr than suggested at first glance by the corrected values presented in this study. This is undoubtably also true for other heavy metals accumulated by earthworms (Macdonald, 1983; Beyer, 1981).

Macdonald (1983) discussed the predation on earthworms by a variety of animals, including birds, foxes, moles and some amphibians. He indicated that some of these species could consume as many as 40 (average of 20) individual earthworms during a single foraging session. If the earthworms are contaminated with Cr the potential exists for considerable accumulation of Cr(III) in the predators. In this manner Cr(III) could move through the food web. The predators could themselves become prey for other animals or could die from chronic overexposure to Cr. This is not likely to be a widespread problem, especially given the localized nature of most Cr contamination. However, many contaminated industrial areas are reclaimed as parks and wildlife habitat, areas in which people deliberately cultivate wildlife. Under some circumstances this wildlife could be exposed to considerable amounts of Cr(III) via the earthworm population.

4.3 Effects of Chromium on Earthworm Activity

Earthworms are known to have a variety of physical, biological and chemical effects upon the soil. Physical effects include changes in porosity, aggregate formation and stabilization as well as mixing of organic and mineral layers. Changes in microbial biomass and population structure, respiration, nitrification and N mineralization can be included amongst those classified as biological changes induced by earthworm activity. Chemical effects commonly attributed to earthworms are changes in pH (particularly in acidic LFH layers), elemental cycling of nutrients such as C, N, P and Ca and possibly of other elements such as Pb. Organic matter decomposition is also obviously affected by earthworms. Some or all of these factors could potentially be examined to further estimate the influence of heavy metals, such as Cr, on earthworms.

Given the length of the study it was felt that consideration of physical changes produced in soil by earthworms would be unwarranted. Since a geophagous species was used, rates of organic matter decomposition were also unlikely to change significantly over the course of the experiment. The factors examined as indicators of the effects of Cr on *E. fetida* were: microbial respiration, mineral N, biomass C and N, and the amount of casts and

cocoons produced. Cast and cocoon data were insufficient for quantitative analysis; qualitative observations will be reported.

The CFI technique (Jenkinson and Powlson, 1976) is commonly used to estimate biomass C and N. When examined, the variability of the fumigated CO_2-C data was found to be very high, leading to questions concerning the validity of calculating biomass C. One possible reason for the high variability is that additions of Cr one day to two weeks prior to fumigation kill some of the biomass, resulting in a flush similar to that found when fresh substrate is added. Jenkinson and Powlson (1976) found that fresh substrate added shortly before fumigation produces inaccurate biomass estimations. Brookes *et al.*(1986) determined that the CFI technique is suitable for soils contaminated with low to moderate amounts of metals either many years prior to measurement or immediately following fumigation (and just before incubation). They did not examine soils where the metal had been added shortly prior to fumigation (i.e. one to six weeks versus 20 years), nor did they consider metal additions on the order of those used in this study (~ 10 times those used by Brookes *et al.*, 1986). Both of these factors might alter the k_c and/or the response to fumigation and therefore might result in inaccurate biomass estimation. Because of this uncertainty and the previously mentioned variability, only respiration (unfumigated CO_2-C evolved) values are discussed.

Similar concerns arose with respect to biomass N. The value of k_n is calculated from the relationship of C to N in microbial populations (Shen *et al.*, 1984) or directly from the NH_4-N measurement (Voroney and Paul, 1984). In either case, k_n is affected by the microbial C to N ratio. Shen *et al.* (1984) caution that their calculation will not be valid for soils where the proportions of the various biomass constituents are abnormal (and hence C:N is altered). Voroney and Paul (1984) state that their k_n values are only valid for soils with C_F:N_F ratios in the same range as in their study (C_F:N_F is the ratio of CO_2-C evolved : net NH_4-N accumulated during fumigation and incubation). Their C_F:N_F ratio will also be dependent on the nature of the microbial population. The addition of Cr to the soil undoubtably alters the proportions of biomass constituents. Fungi are considerably more resistant to Cr toxicity than bacteria (Zibilski and Wagner, 1982; Beck, 1981); autotrophs are less sensitive than

heterotrophs (James and Bartlett, 1984b). Some species of fungi are more resistant to Cr than others (Babich *et al.*, 1982) and gram negative bacteria are completely inhibited at much lower Cr application rates than gram positive species (Ross *et al.*, 1981). These differences in resistance to Cr indicate that the C_F:N_F ratio is quite probably significantly altered in Cr contaminated soils. For this reason the levels of mineral N were examined rather than calculating biomass N. Data for time zero NH_4-N and NO_3-N levels were not available so the flush of N over the incubation period (net mineral N) cannot be calculated. Absolute changes in mineral N over the course of the experiment cannot therefore be discussed but relative comparisons between treatments are still valid.

The two earthworm treatments E1 and E2 appear to influence microbial activity differently. The earthworms added immediately following Cr (E1) resulted in greater microbial respiration and mineral N than either the control or the earthworms added after a two day lag (E2) (Table 2). This suggests that the addition of *E. fetida* immediately following Cr had an ameliorating influence on the microbial population. As expected, the no-Cr treatment had higher respiration than either of the two Cr treatments, although the effect of Cr(III) on respiration was much less than that of Cr(VI). Mineral N levels are much higher for Cr(VI) than for either Cr(III) or the control (Table 2).

When the Cr treatment, earthworm treatment interactions are considered the effects of Cr on *E. fetida* activity become clearer. From Figure 13 it can be seen that Cr(VI) suppressed respiration equally, irrespective of whether earthworms were present. In contrast, the Cr(III) treatment, while statistically less than the control, exhibits the same trend as the control, with respiration greater for earthworms added immediately than either of the other two treatments. These results suggest two things. First, Cr(VI) affects *E. fetida* activity sufficiently to produce no difference in respiration between earthworm and non-earthworm treatments. Death of earthworms at later incubation dates was thought to be a possible cause but removal of these data points did not significantly alter the means for Cr E1-Cr(VI) and E2-Cr(VI) treatments (data not presented). Second, when the earthworms are added to Cr(III) treated soil, their influence is only slightly altered from that of controls.

Table 2: ANOVA Results for the Variables Respiration and Mineral N

Respiration (CO_2-C evolved, µg/g)	Total Unfumigated-N (µg/g)
E1 >> NW > E2	E1 > E2 > NW
A B C	A B C
no Cr > Cr(III) >> Cr(VI)	Cr(VI) >> Cr(III) = no Cr
A B C	A B B

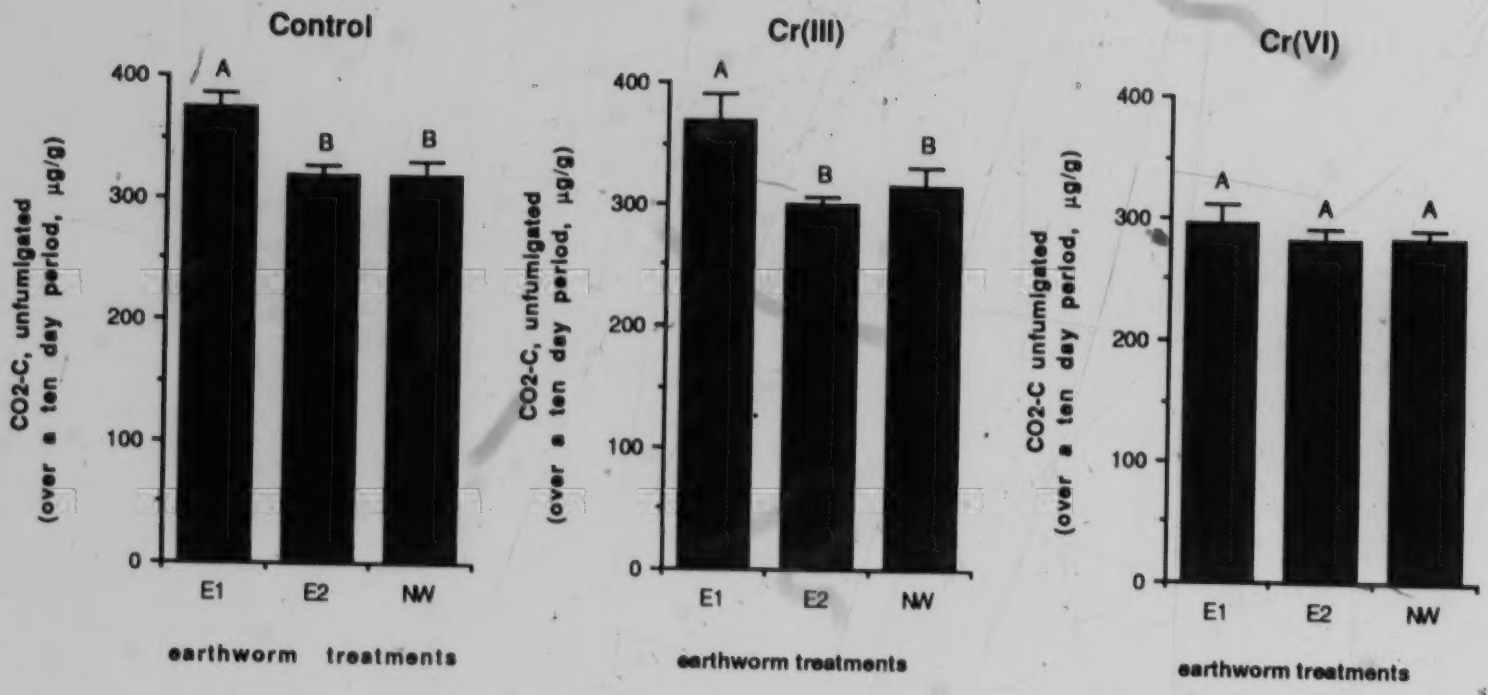


Figure 13: Respiration for E1, E2 and NW treatments within Cr treatments

In some fashion, earthworms added immediately after Cr are able to mitigate the effects of Cr(III) on the microbial population.

Not only did earthworms added two days following Cr(III) (E2) not have any influence on respiration, E2 control earthworms also did not alter respiration relative to non-worm controls (Figure 13). This suggests that the E2 treatment had no effect on the microbial population at all, relative to NW. However, examination of the changes in respiration over time for each of the treatments show that this is not the case (Figure 14 a,b,c). In general, the changes in respiration over time are similar within each earthworm treatment, irrespective of Cr treatment; that is, differences occur between earthworm treatments. E1, no-Cr and Cr(III) treatments (Figure 14 a) have significantly higher early respiration levels (as compared to E2 and NW) which fluctuate over the first eight days and then decline, reaching levels similar to that of E2 and NW treatments by 16 days. The E1-Cr(VI) treatment follows the same trend but respiration levels are significantly lower. E2 treatments showed a small decline in respiration, followed by an increase to levels higher than at day one, followed by a further decline (Figure 14 b). NW treatments (Figure 14 c) showed an initial sharp decline with recovery beginning between eight and 16 days and levelling off after 32 days. While following the same general patterns Cr(VI) treatments were lower than Cr(III) or no-Cr across all three earthworm treatments, as also indicated in Figure 13 and Table 2.

Earthworm treatments appear to dictate respiration patterns over time but not overall respiration levels, which are significantly decreased by Cr(VI). The differences between the two earthworm treatments, E1 and E2, suggest a combination of effects. With the exception of the fluctuations in respiration over days one through eight in E1, and the higher initial levels, both E1 and E2 treatments show a decline in respiration over time (Figure 14 a,b). This decline occurs in all earthworm-Cr treatment combinations but not in NW treatments, indicating that it is earthworm derived. If one makes the assumption that *E. fetida* has a preference for microbial material then this decline in respiration could be due to a decrease in population due to predation. The disturbance of the soil by *E. fetida* will also kill some biomass but this is probably more than balanced by the generation of new biomass in

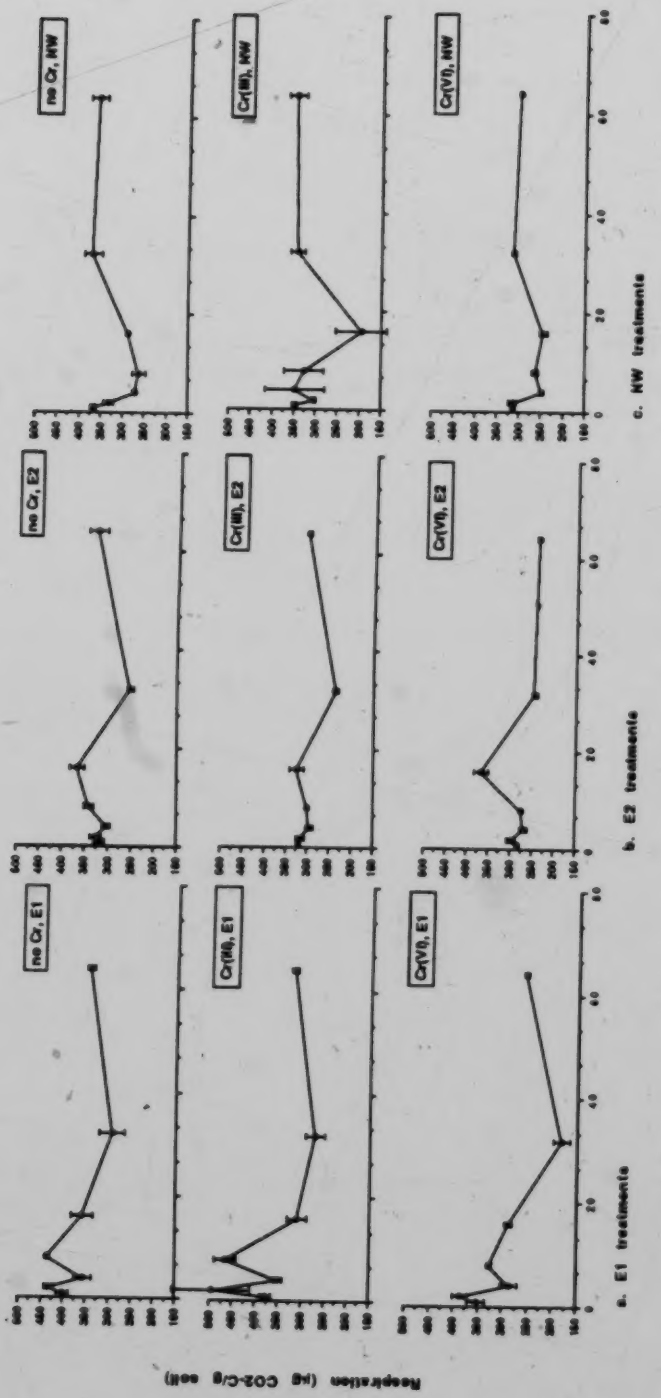


Figure 14: Changes in respiration over time for all treatment combinations

casts and in freshly exposed soil organic matter (Scheu, 1987a; Shaw and Pawluk, 1986b; Lee, 1985; Satchell, 1983). The levelling off of respiration might be due to the achievement of a balance between earthworm consumption of microbial biomass, loss of biomass due to disturbance, and the generation of new biomass.

The high initial levels of respiration in E1 do not continue over time, but are significantly higher than E2 respiration levels. The two day lag between Cr application and earthworm introduction is therefore enough time for respiration to decrease, due to the presence of Cr and/or the disturbance of the soil when loading columns. Once earthworms are added (either immediately or after a time lag) the biomass initially recovers and then declines. A possible sequence for these changes in respiration is as follows: a) An initial increase in respiration due to increased available organic matter as a result of bioturbation and cast production, followed by b), a decline in respiration due to the rate of predation by earthworms exceeding the rate of new biomass generation, followed by c), the achievement of a balance between processes in a) and b) and the stabilization of respiration at a given level. In non-worm treatments, only the disturbance due to loading and/or Cr addition occurs. This sequence could explain the changes over time in respiration but not the levels. The relative level which respiration eventually achieves is determined in this case by chromium. Cr(VI) significantly depresses respiration relative to the controls, while respiration levels under Cr(III) are slightly but not significantly lower than controls.

Mineral N values also illustrate the influence of Cr(VI) on earthworms. Cr(VI) mineral N is significantly higher than either Cr(III) or the control (Table 2). Within all three Cr treatments, E1 had higher mineral N than E2 or NW (Figure 15). Both E1 and E2 for Cr(VI) have significantly more mineral N than any other treatment. A possible reason for this is the release of N upon the death of earthworms. A rough calculation, based on earthworms containing approximately 12 % N by dry weight (Lee, 1985) and an average dry weight for *E. fetida* of 0.0940 g, revealed that 17-23 $\mu g\ N\ g^{-1}$ soil could be released upon the death of one worm or 121 - 162 $\mu g\ N\ g^{-1}$ soil if all seven worms in the column died (as was the case for some E1 and E2, 32 and 64 day treatments). This amount of nitrogen is enough to significantly

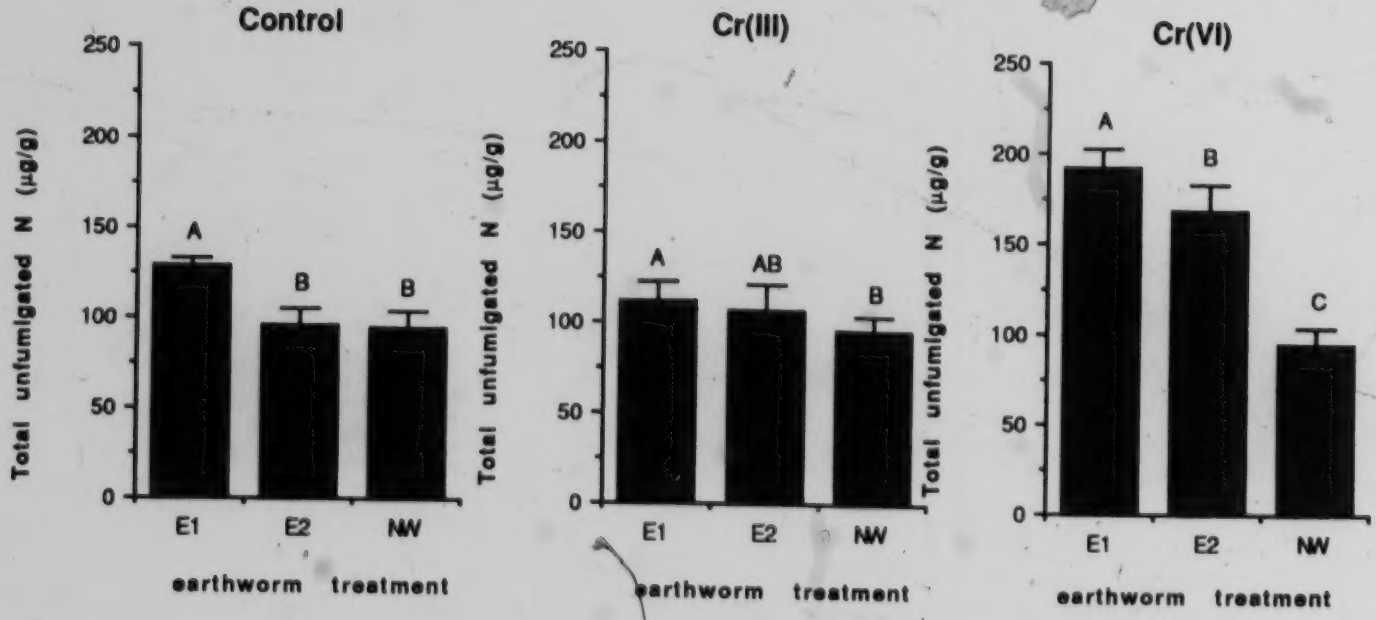


Figure 15: Total Unfumigated Nitrogen, for E1, E2 and NW treatments within Cr treatments

increase mineral N levels in the affected treatments. Not all individual earthworms that died were decomposed, only those where death and initial decomposition occurred within the 72 h checking time.

Figure 16 a,b and c illustrates the changes in mineral N over time for each treatment combination. The higher overall mineral N levels for E1 and E2 Cr(VI) treatments are reflected in the increasing N between one and 16 days and in the higher 64 d levels relative to controls and non-worm treatments. The influence of live earthworms can be seen in the Cr(III) treatments (No earthworms that died in these treatments were decomposed). Mineral N levels at the beginning of the incubation are equal to or lower than control or non-worm treatments but by 32 d are higher, and remain so for the duration of the incubation. There are two possible reasons for this increase in mineral N: 1) The increase in mineral N corresponds to a decline in respiration (see Figure 14 a,b), i.e. N in "mineral" form was released as the microbial population decreased, or 2) increased mineral N is released as a result of increased microbial population turnover occurring in freshly exposed organic matter and/or casts.

Scheu (1987b) found that increased mineral N was directly related to the biomass of worms present and not to N release by earthworm death or excretion. The majority of the increased mineral N was found in casts indicating that passage of the material through the gut was the cause, direct or indirect, of changes in mineral N. Scheu (1987a) also reported an 86 % increase over time in respiration in casts over that of soil and attributed both increased respiration and increased N mineralization to an increase in the microbial biomass in the casts. In this study respiration declined over time while mineral N increased, suggesting that while explanation 2) above is more likely to hold in a natural system such as studied by Scheu, explanation 1) is more plausible for a metal contaminated system. Whether or not the observed changes in mineral N over time are due to the direct effects of Cr and/or to changes in earthworm activity is not clear.

In any event, the response of the microbial population, as reflected in changes in respiration and mineral N, to the activity of earthworms is indirect (Andersen *et al.*, 1985). Changes occur over time gradually, as

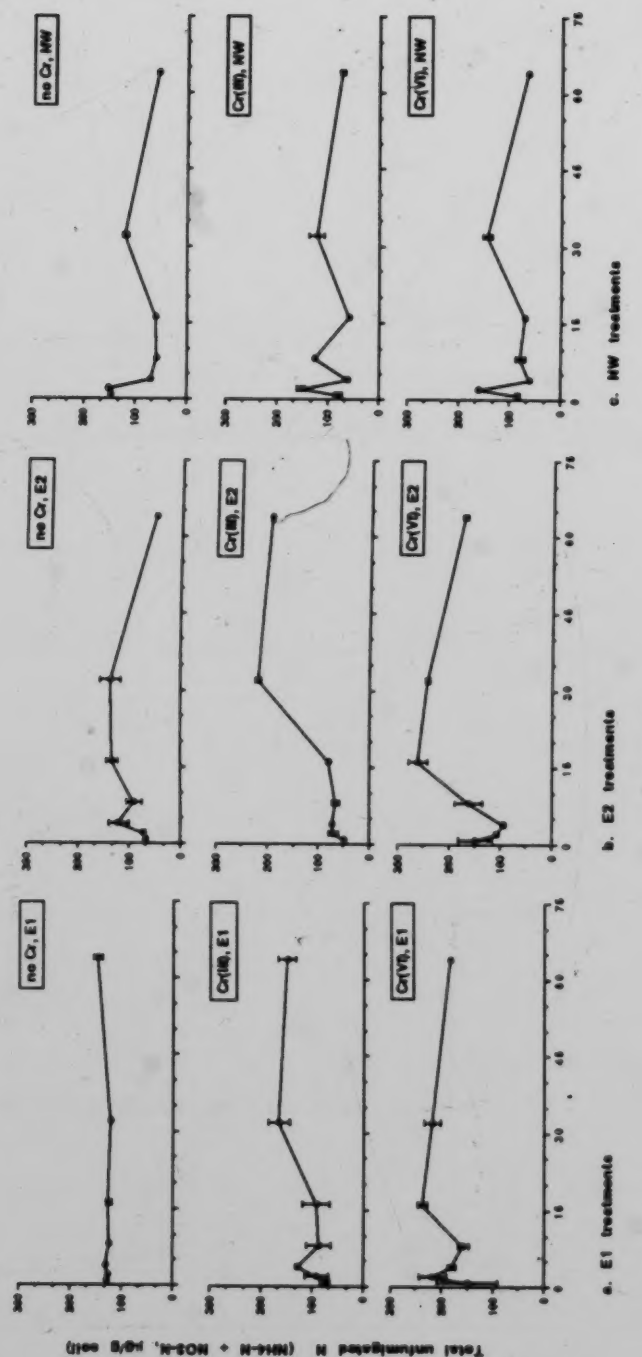


Figure 16: Changes in total unfumigated N over time for all treatment combinations

opposed to rapid, step-wise fashion, as environmental conditions change. Anderson *et al.* (1985) noted the very important but indirect influence of a variety of soil invertebrates, including earthworms, on nitrogen cycling. Lumbricid worms are usually quantitatively the most important group of soil invertebrates involved in both C and N mineralization (Bengtsson *et al.*, 1988).

Although insufficient data were collected for quantitative analyses of cocoon and cast amounts, qualitative observations were made. Casts were most abundant in control columns, with the soil appearing to be almost entirely worked over by the end of the 64d incubation. The least amount of casts occurred in the Cr(VI) treatments, with large areas of the column appearing to be untouched even by the end of the incubation. Cr(III) treatments had more casts than Cr(VI) but still markedly less than controls. Live worms removed from later date Cr(III) and Cr(VI) columns (16, 32, 64 d) looked smaller than control worms from the same dates. Examination of the freeze dried earthworm weights gave an average weight of 0.095, 0.055 and 0.047 g worm^{-1} for controls, Cr(III) and Cr(VI) treatments respectively. Cocoon numbers followed a similar pattern to that shown by the casts, with none or very few cocoons seen in later Cr treatments.

These observations agree with the previously presented data, and indicate that both trivalent and hexavalent chromium have adverse effects on *E. fetida*. Hartenstein *et al.*, (1979) found that cocoon production was affected by population density, temperature, pH, moisture and food source. Maximum cocoon production was achieved using a mixture of mineral soil and sewage sludge; soils very high in organic matter were more productive than the sludge alone but less than the mixture. Hartenstein *et al.* (1979) and Lee (1985) clearly indicate that adverse environmental conditions will cause a decline in cocoon production. In subsequent studies, Hartenstein *et al.* (1981b) also showed that exposure to some metals, particularly Cd, Cu and Ni, resulted in reduced biomass, reduced growth rates and in some cases, death. Given that factors such as temperature, pH, moisture and population density were controlled and that the control earthworms survived well on the food source provided, the presence of Cr is the most plausible cause for the observed reduction in biomass and in cocoon and cast numbers.

4.4 Effects of *Eisenia fetida* on Chromium Chemistry.

The presence of earthworms altered the solution chemistry of Cr. With the exception of the water extractable Cr(VI) values for one treatment combination, solution Cr levels for NW always exceeded those for E1 or E2 in Cr treatments (Table 3). As would be expected, there is no difference between earthworm and non-worm treatments where no Cr was added. For Cr(III) treatments, the pattern is consistent for all soluble Cr measures: NW > E1 = E2, that is, no difference existed between the E1 and E2 treatments. There is no such pattern to the Cr(VI) treatments. In general, differences between E1 and E2 are smaller than between those treatments and NW. The exception is the water extractable Cr(VI) values for the Cr(VI) treatment, where E2 is larger than NW and both are considerably larger than E1. High variability within these particular treatment means makes them suspect.

While these results are far from conclusive, they do indicate that *E. fetida* has an influence on the soil-Cr chemistry. The influence is indirect and due to the earthworms' physical alteration of the soil. Commutation of soil mineral and organic matter while passing through the gut would increase the surface area exposed to chromium and thus result in greater sorption of both Cr species. The gut conditions themselves might also contribute to the greater sorption of Cr by the soil. The pH of the earthworm gut varies from 6.4 to 7.5 depending on the portion of the gut measured and the species involved (Lavarack, 1963). These data are not for *E. fetida* specifically, but in combination with evidence that the earthworm gut is an anaerobic environment (Shaw and Pawluk, 1986b; Lee, 1985), it suggests a microclimate conducive to the organic complexation of Cr. Assuming that *E. fetida* preferentially consumes organic rich portions of the soil, the gut might well provide a situation where Cr and organic matter are in close proximity to each other. This could result in greater complexation and/or reduction of Cr by organic matter (and subsequent sorption onto soil) than would normally occur in the soil. An alternate explanation is that the earthworms themselves absorbed sufficient Cr to reduce solution levels. This is unlikely,however, as Cr solubility is strongly controlled by sorbed phases and any Cr taken out of solution by the earthworms would be replaced, all other

Table 3: Means for various solution Cr measures for earthworm-Cr treatment combinations, shown by Cr treatment.

	Cr treatments		
Solution Cr parameters	no Cr	Cr(III)	Cr(VI)
H_2O Cr(III) + Cr(VI)	NW = E1 = E2 a a a	NW >> E1 = E2 a b b	NW > E1 = E2 a b b
H_2O Cr(VI)	NW = E1 = E2 a a a	NW > E1 = E2 a b b	E2 > NW >> E1 a b c
K_2HPO_4 Cr(III) + Cr(VI)	NW = E1 = E2 a a a	NW > E1 = E2 a b b	NW > E1 ≥ E2 a b c
K_2HPO_4 Cr(VI)	NW = E1 = E2 a a a	NW > E1 = E2 a b b	NW > E1 > E2 a b c

E1 = Earthworms added immediately after Cr application, E2 = Earthworms added two days after Cr application, NW = no earthworms. a, b, and c indicate means are significantly different, P≤ 0.05.

factors remaining equal. The increased physical proximity of the reaction constituents due to a combination of physical movement and alteration of the soil, and the unique environment provided by the earthworm gut, is a more likely explanation. Without further study, it is sufficient to say that the presence of earthworms does appear to alter the soil-Cr chemistry.

CHAPTER FIVE: SUMMARY AND CONCLUSIONS

The data presented clearly show that Cr in both oxidation states affects *E. fetida* in a variety of ways. Results presented in the previous chapter can be summarized as follows:

1. *E. fetida* accumulates Cr from Cr(VI) and Cr(III) loaded soils.

2. Accumulation of Cr is greater (by an order of magnitude) from Cr(III) treatments than from Cr(VI) treatments.

3. Increasing the level of Cr(III) application does not affect accumulation but increasing the level of Cr(VI) application does increase Cr accumulation.

4. Increased length of exposure to Cr increases accumulation for both Cr(III) and Cr(VI).

5. In this soil, applied Cr(III) is toxic at levels greater than 1500 ppm while applied Cr(VI) is toxic at 75 ppm.

6. Respiration and mineral N levels decrease with the application of either Cr(III) or Cr(VI). The effects of Cr(III) on the microbial population are ameliorated somewhat by the presence of earthworms.

The first five points in the above list apply generally to Objectives 1,2 and 3 discussed in the Introduction. Point six refers to Objective Four, to determine if the type and form of Cr influences earthworm activity.

While *E. fetida* accumulates Cr from both Cr(VI) and Cr(III) treatments, it does so in varying amounts depending on the length of exposure and the level of application. Cr is accumulated from the Cr(III) treatments by *E. fetida* in increasing amounts over time but not as the level of application increases. Cr from Cr(VI) treatments is likely increasingly accumulated over time, but toxicity of Cr(VI) to *E. fetida* prevented a definite answer. In contrast to Cr(III) treatments, *E. fetida* does accumulate increasing

amounts of Cr from Cr(VI) treatments as the level of application is increased. That Cr from Cr(VI) treatments is increasingly accumulated by *E. fetida* both over time and as application levels increase is consistent with the known chemistry of Cr(VI). While much Cr(VI) added to soil will be reduced or absorbed, the rate of both of these processes is slower than the precipitation of Cr(III) added to soil (Rai *et al.*, 1988). The capacity of a mineral soil to "fix" Cr(VI) by either process is perhaps also more limited than the same soil's capacity to sorb Cr(III). This is also confirmed by the adsorption data, which indicated that far more Cr was found in solution in Cr(VI) treated soil than in Cr(III) treated soil In any event, Cr(VI) is generally both more soluble and mobile in soil than Cr(III) and is perhaps therefore also more omnipresent in an earthworm's diet. Cr(VI) is also more readily taken up by earthworms than is Cr(III), due to its ability to cross cell membranes. This fact and Cr(VI)'s tendency to poison enzymes partially explain its greater toxicity to *E. fetida*.

Why Cr(III) should be increasingly accumulated over time but not with increased application rate is perhaps, at first glance, unclear. If *E. fetida* had a maximum capacity for Cr(III) one might expect the accumulation of Cr, as the Cr(III) application level increased, to initially increase and then level off. This does not occur. Nor is the amount accumulated with increased Cr(III) application level close to earthworm Cr levels accumulated over time. Examination of the relationship between %kill and Cr solution values showed no correlation. The amount of variation in %kill was only poorly explained by non-recoverable Cr. If Cr(III)-soil chemistry is accounted for, however, a possible explanation exists. Increasing the Cr(III) application level will not increase Cr(III) in soil solution once the solubility controlling solid level of Cr(III) is reached. Only more solid $Cr(OH)_{3(ss)}$ or $Fe,Cr(OH)_{3(ss)}$ will form. The length of exposure of the earthworm to the remaining Cr in solution and to sorbed complexes thus becomes the dominant factor in determining Cr accumulation by *E. fetida*.

The presence or absence of Cr appeared to determine the levels of respiration obtained in any given treatment combination but not the pattern of change over time. Again, the type of Cr is important, with Cr(VI) depressing respiration significantly more than Cr(III). The presence of earthworms results in the apparent stabilization of respiration sooner and at

higher levels than in non-worm controls. The effects of both earthworms and Cr also were apparent in changes in mineral N levels. As expected Cr(VI) adversely affected mineral N more than did Cr(III). Earthworms added immediately after Cr (E1) resulted in higher mineral N levels than earthworms added after two days (E2). The changes in both respiration and mineral N were gradual and although significant were not large. This is in keeping with previous observations that the effect of earthworms on microbial populations is indirect (Andersen *et al.*, 1985). The qualitative observations of a decline in cocoon and cast amounts and also in earthworm biomass for both Cr treatments is also confirmed by the literature.

The accumulation of both Cr(III) and Cr(VI) by *E. fetida* shown in this study stands in contrast to the work of others. Helmke *et al.* (1979) found no accumulation of Cr and concluded that Cr in sludge is biologically unavailable to earthworms. Hartenstein *et al.* (1981) found both Cr and Pb to be innocuous to *E. fetida* even at extremely high concentration, but were careful to note that this may only hold for metals in organic rich materials such as sewage sludge. However, studies using mineral soils and Cr also suggested that Cr was not readily bioavailable (Ma, 1982). Obviously then, the use of a mineral soil in this study cannot entirely account for the discrepancies in observed toxicity between this study and others. The fact that most of the studies discussed above used only trivalent chromium (or did not specify which oxidation state was used) is also not sufficient to account for the differences in toxicity. Granted, Cr(III) is less toxic than Cr(VI) in this study, by roughly an order of magnitude, but Cr(III) levels sufficient to kill 50 % of *E. fetida* in this study (~ 4000 ppm) are themselves an order of magnitude less than levels reported to be completely innocuous by Hartenstein *et al.* (1981b, 46,000 ppm).

These conflicting results emphasize the need to know more than just the total amount of metal present. The chemical form taken by the metal in the soil obviously influences its toxicity to earthworms, an observation which confirms previous work by Hartenstein *et al.* (1981a,b) and Beyer and Cromartie (1987) with metals other than Cr. Examination of various forms of Cr does not in this case provide any further explanations. There appears to be no connection between solution levels of Cr and toxicity.

That Cr(III) is less toxic to *E. fetida* than Cr(VI) is not surprising, in light of the literature concerning the biochemical action of both oxidation states to other organisms (see discussion in Literature Review). Perhaps because Cr(VI) has been a greater health concern to humans and because until recently Cr(III) was assumed to be inert and immobile in soil, little attention had been paid to the possible consequences of long term exposure of soil fauna to Cr(III). This study has clearly shown that Cr(III) can accumulate in *E. fetida* over time and potentially spread out through the food web. It must also be remembered that animals consuming the earthworms will consume greater amounts of Cr than measured here due to their consumption of the gut material containing Cr. Beyer (1981) suggested that in some cases this could result in a significant increase in the amount of metal consumed by the predator. This appears also to be the case in this study.

CHAPTER SIX: REFERENCES

Adriano, D.C. 1986.
"Chromium." Chap. 5, In. Trace Elements in the Terrestrial Environment. Springer - Verlag. New York, N.Y.

Andersen, C. 1979.
Cadmium, lead and calcium content, number and biomass, in earthworms (Lumbricidae) from sewage sludge treated soil. Pedobiologia. 19: 309-319.

Andersen, C. and J. Laursen. 1982.
Distribution of heavy metals in *Lumbricus terrestris, Aporrectodea longa* and *A. rosea* measured by atomic absorption and X-ray fluorescence spectrometry. Pedobiologia. 24: 347-356.

Anderson, J.M., M.A. Leonard, P. Ineson and S. Huish. 1985.
Faunal biomass: a key component of a general model of nitrogen mineralization. Soil Biol. Biochem. 17(5): 735-737.

Babich, H., M. Shiffenbauer and G. Stotzky. 1982.
Comparitive toxicity of trivalent and hexavalent chromium to fungi. Bull. of Envirn. Contamn. and Toxicology. 28: 452-459.

Bartlett, R.J. 1986.
Chromium oxidation in soils and water: measurements and mechanisms. In. Proceedings, Chromium Symposium 1986: an update. (D.M. Serrone, ed.) Industrial Health Foundation., Pittsburg PA. pp. 310-330.

Bartlett, R.J. and B.R. James. 1979.
Behavior of chromium in soils: III. Oxidation. J. Environ. Qual. 8(1): 31-35.

Bartlett, R.J. and J.M. Kimble. 1976a.
Behavior of chromium in soils: I. Trivalent Forms. J. Environ. Qual 5(4): 370-383.

Bartlett, R.J. and J.M. Kimble. 1976b.
Behavior of chromium in soils: II. Hexavalent forms. J. Environ. Qual. 5(4): 383-386.

Beck, T. 1981.
The toxic effects of heavy metals commonly found in urban wastes on the soil micro flora. Z. Pflanzenernaehr Bodenkd. 144(6): 613-627.

Bengtsson, G., M. Berden and S. Rundgren. 1988.
Influence of soil animals and metals on decomposition processes: a microcosm experiment. J. Environ. Qual. 17:113-119.

Beveridge, T.J. 1984.
Mechanisms of the binding of metallic ions to bacterial walls and the possible impact on microbial ecology. In. Current perspectives in microbial ecology. M.J. Klug and C.A. Reddy (eds.) Am. Soc. for Microbiol. pp. 601-608.

Beyer, W.N. 1981.
Metals and terrestrial earthworms (Annelida: Oligocheata). In. Workshop on the role of earthworms in the stabilization of organic residues. Vol. 1., Proceedings. Beech Leaf Press, Kalamazoo, MH. pp. 137-150.

Beyer, W.N. and E.J. Cromartie. 1987.
A survey of Pb, Cu, Zn, Cd, Cr, As and Se in earthworms and soil from diverse sites. Environ. Mon. & Assessment. 8:27-36.

Beyer, W.N., G. Hensler and J. Moore. 1987.
Relation of pH and other soil variables to concentrations of Pb, Cu, Zn, Cd and Se in earthworms. Pedobiologia. 30:167-172.

Bolton, P.J. and J. Phillipson. 1976.
Burrowing, feeding, egestion and energy budgets of *Allolobophora rosea* (Savigny) (Lumbricidae). Oecologia (Berl.) 23: 225-245.

Bouche, M.B. 1977.
"Stratégies Lombriciennes." In. Soil Organisms as Components of Ecosystems. Proc. 6th Int. Coll. Soil Zool. U. Lohm and T. Persson (eds.). Ecol. Bull. (Stockholm) 25: 122-132.

Bouche, M.B. 1985.
"Foreward." In. Earthworms: their ecology and relationships with soils and land use. K.E. Lee. Academic Press, Sydney, Australia. pp. ix.

Bowman, W.S., G.H. Faye, R. Saturno, J.A. McKeague and H. Kodama. 1979.
New CCRMP reference soils SO-1 to SO-4. Geostandards Newsletter. 3(2): 109-113.

Breeze, V.G. 1973.
Land reclamation and river pollution problems in the Croal Valley caused by waste from chromate manufacture. J. Applied Ecology. 10: 513-525.

Brookes, P.C., C.E. Heijnen, S.P. McGrath and E.D. Vance. 1986.
Soil microbial biomass estimates in soils contaminated with metals. Soil Biol. Biochem. 18(4): 383-388.

Carter, A., E.A. Kenney, T.F. Guthrie and H. Timmenga. 1983.
"Heavy metals in earthworms in non-contaminated and contaminated agricultural soil from near Vancouver, Canada." In. Earthworm Ecology: from Darwin to vermiculture. J.E. Satchell (ed.) Chapman and Hall, London, U.K. pp. 267-274.

Cary, E.E., W.H. Allaway and O.E. Olson. 1977.
Control of chromium concentration in food plants I. Absorption and translocation of Cr by plants. J. Agric. Food Chem. 25(2): 300-304.

Chang, F.-H. and F.E. Broadbent. 1982.
Influence of trace metals on some soil nitrogen transformations. J. Environ. Qual. 11(1): 1-4.

Curry, J.P. and D.E.F. Cotton. 1983.
"Earthworms and land reclamation." In. Earthworm Ecology: from Darwin to vermiculture. J.E. Satchell (ed.) Chapman and Hall, London, U.K. pp. 215-228.

Darwin, Charles. 1881.
The formation of vegetable mould through the action of worms with observations on their habits. Murray, London. 298 pp.

Dudas, M.J. and S. Pawluk. 1970.
Chernozem soils of the Alberta Parklands. Geoderma 3: 19-35.

Eary, L.E. and D. Rai. 1987.
Kinetics of Chromium(III) oxidation to Chromium(VI) by reaction with manganese dioxide. Environ. Sci. & Tech. 21(2):1187-1193.

Fleming, T.P. and K.S. Richards. 1981.
Localization of adsorbed heavy metals on the earthworm body surface and their retrieval by chelation. Pedobiologia. 23:415-418.

Gish, C.D. and R.E. Christensen. 1973.
Cd, Ni, Pb and Zn in earthworms from roadside soil. Envtl. Sci. Tech. 7: 1060-1062.

Graff, O. 1983.
"Darwin on earthworms - the contemporary background and what the critics thought." In. Earthworm Ecology: from Darwin to vermiculture. J.E. Satchell (ed.) Chapman and Hall, London, U.K. pp. 5-18.

Griffen, R.A., A.K. Au and R.R. Frost. 1977.
Effect of pH on adsorption of chromium from landfill - leachate by clay minerals. J. Envtl. Sci. Health, A. 12(8):431-449.

Grove, J.H. and B.G. Ellis. 1980b.
Extractable iron and manganese as related to soil pH and applied chromium. SSSAJ. 44: 243-246.

Guerrero, R.D. 1983.
"The culture and use of *Perionyx excavatus* as a protein resource in the Phillipines." In. Earthworm Ecology: from Darwin to vermiculture. J.E. Satchell (ed.) Chapman and Hall, London, U.K. pp. 309-314.

Hartenstein, F., E. Hartenstein and R. Hartenstein. 1981b.
Gut load and transit time in the earthworm *Eisenia foetida*. Pedobiologia. 22: 5-20.

Hartenstein, R. E.F. Neuhauser and J. Collier. 1980.
Accumulation of heavy metals in the earthworm *Eisenia foetida*. J. Environ. Qual. 9(1): 23-26.

Hartenstein, R., E.F. Neuhauser and A. Narahara. 1981a.
Effects of heavy metals and other elemental additives to activated sludge on growth of *Eisenia foetida*. J. Environ.Qual. 19(3): 372-376.

Hartenstein, R., E.H. Neuhauser and D.L. Kaplan. 1979.
Reproductive potential of the earthworm *Eisenia foetida*. Oecologia. (Berl.) 43: 329-340.

Hartford, W.H. 1986.
"The fundamental chemistry of industrial chromium compounds, and some thoughts on their biological activity." In. Proceedings. Chromium symposium 1986: an update. D.M. Serrone (ed.) Industrial Health Foundation, Inc. Pittsburg, PA. pp. 9-41.

Hatanaka,K., Y. Ishioka and E. Furuichi. 1983.
"Cultivation of *Eisenia fetida* using dairy waste sludge cake." In. Earthworm Ecology: from Darwin to vermiculture. J.E. Satchell (ed.) Chapman and Hall, London, U.K. pp. 323-330.

Helmke, P.A., W.P. Robarge, R.L. Korotev and P.J. Schomberg. 1979.
Effects of soil - applied sewage sludge on concentrations of elements in earthworms. J. Environ. Qual. 8(3):322-327.

Huffman, E.W.D. and W.H. Allaway. 1973.
Chromium in plants: distribution to tissues, organelles and extracts and availability of bean leaf Cr to animals. J. Agr. Food Chem. 21(6): 982-986.

Ireland, M.P. 1975.
The effect of the earthworm *Dendrobaena rubida* on the solubility of lead, zinc and calcium in heavy metal contaminated soils in Wales. J. Soil Science. 26: 313-318.

Ireland, M.P. 1979.
Metal accumulation by the earthworms *Lumbricus rubellus, Dendrobaena veneta* and *Eiseniella tetraedra* living in heavy metal polluted sites. Environmental Pollution. 19: 201-206.

Ireland, M.P. 1983.
"Heavy metal uptake and tissue distribution in earthworms." In. Earthworm Ecology: from Darwin to vermiculture. J.E. Satchell (ed.) Chapman and Hall, London, U.K. pp. 247-265.

James, B.R. and R.J. Bartlett. 1983a.
Behavior of chromium in soils:V. Fate of organically complexed Cr(III) added to soil. J. Environ. Qual. 12(2): 169-172.

James, B.R. and R.J. Bartlett. 1983b.
Behavior of chromium in soils:VI. Interactions between oxidation - reduction and organic complexation.J.Environ.Qual.12(2):173-176.

James, B.R. and R.J. Bartlett. 1983c.
Behavior of chromium in soils: VII. Adsorption and reduction of hexavalent forms. J. Environ. Qual. 12(2):177-181.

James, B.R. and R.J. Bartlett. 1984a.
Nitrification in soil suspensions treated with chromium (III,VI) salts or tannery wastes. Soil Biol. Biochem. 16(3): 293-295.

James, B.R. and R.J. Bartlett. 1984b.
Plant - soil interactions of chromium. J. Environ. Qual. 13(1): 67-70.

Jamieson, B.G.M. 1981.
"Chloragocytes." Chapter 4, In. The Ultrastructure of the Oligochaeta. Academic Press, Toronto. pp. 96-118.

Jaworski, J.F. 1984.
Chromium update 1984: Environmental and nutritional effects of Chromium. National Research Council of Canada, NRCC Report No. 23917. Ottawa, Ontario. 56 pp.

Jenkinson, D.S. and D.S. Powlson. 1976.
The effects of biocidal treatments on metabolism in soil - V. a method for measuring soil biomass. Soil Biol. Biochem. 8:209-213.

Juma, N.G. and M.A Tabatabai. 1977.
Effects of trace elements on phosphatase activity in soils. SSSAJ. 41: 343-346.

Keefer, R.F., R.N. Singh and D.J. Horvath. 1986.
Chemical composition of vegetables grown on an agricultural soil amended with sewage sludges. J. Environ. Qual. 15(2): 146-152.

Kladivko, E.J., A.D. Mackay and J.M. Bradford. 1986.
Earthworms as a factor in the reduction of soil crusting. SSSAJ 50:191-196.

Koons, R.D. and P.A. Helmke. 1978.
Neutron activation analysis of standard soils. SSSAJ. 42:237-240.

Korallus, U. 1986.
"Biological activity of Chromium(VI) - against Chromium(III) - compounds: new aspects of biological monitoring." In. Proceedings, Chromium symposium 1986: an update. D.M. Serrone (ed.) Industrial Health Foundation, Inc. Pittsburg, PA. pp. 210-230.

Kreis, B., P. Edwards, G. Cuendet and J. Tarradellas. 1987.
The dynamics of PCBs between earthworm populations and agricultural soils. Pedobiologia. 30:379-388.

Lavavrack, M.S. 1963.
The physiology of earthworms. Pergamon Press Ltd., Oxford U.K. 206 pp.

Lee, K.E. 1985.
Earthworms: their ecology and relationships with soils and land use. Academic Press (Harcourt Brace Jovanovich). Sydney, Australia. 411 pp.

Ma, W-C. 1982.
The influence of soil properties and worm-related factors on the concentration of heavy metals in earthworms. Pedobiologia. 24:109-119.

Ma, W., T. Edelman, I. van Beersum and T. Jans. 1983.
Uptake of cadmium, zinc, lead and copper by earthworms near a zinc-smelting complex: influence of soil pH and organic matter. B. Envtl. Cont. & Tox. 30: 424-427.

Macdonald, D.W. 1983.
"Predation on earthworms by terrestrial vertebrates." In. Earthworm ecology: from Darwin to vermiculture. J.E. Satchell (ed.) Chapman and Hall, London, U.K. pp. 393-414.

Malecki, M.R., E.F. Neuhauser and R.C. Loehr. 1982.
The efects of metals on the growth and reproduction of *Eisenia foetida* (Oligochaeta, Lumbricidae). Pedobiologia. 24:129-137.

Martin, M.H. and P.J. Coughtrey. 1982.
Biological monitoring of heavy metal pollution. Applied Science Publishers. London, U.K. 475 pp.

Mba, C.C. 1983.
"Utilization of *Eudrillus eugeniae* for disposal of cassava peel." In. Earthworm Ecology: from Darwin to vermiculture. J.E. Satchell (ed.) Chapman and Hall, London, U.K. pp. 315-322.

McKeague, J.A.(ed.). 1978.
Manual on soil sampling and methods of analysis. Second Edition. Canadian Society of Soil Science. 212 pp.

McKeague, J.A. and M.S. Wolynetz. 1980.
Background levels of minor elements in some Canadian soils. Geoderma. 24: 299-307.

McKenzie, R.M. 1977.
"Manganese oxides and hydroxides." In. Minerals in soil environments. J.B. Dixon and S.B. Weed (eds.) Soil Sci. Soc. Am. Madison, WI pp.181-191.

Mertz, W. 1969.
Chromium occurrence and function in biological systems. Physiol. Rev. 49: 165-239.

Mertz, W. 1979.
"Chromium - an overview." In. Chromium in Nutrition and Metabolism. D. Shapcott and J. Hubert (eds.). Proceedings of the Symposium on Chromium in Nutrition and Metabolism, Sherbrooke Que., Elsevier/North-Holland Biomedical Press, Amsterdam. pp. 1-14.

Mitchell, M.J., C.M. Parkinson, W.E. Hamilton and D.L. Dindal. 1982.
Role of the earthworm *Eisenia foetida* in affecting organic matter decomposition in microcosms of sludge amended soil. I. Applied Ecol. 19: 805-812.

Nakamura, Y. 1982.
Colonization by earthworms of NIIMI waste water treatment trenches. Pedobiologia. 23:399-402.

National Research Council of Canada. 1976.
Effects of chromium in the Canadian environment. National Research Council of Canada, NRCC Report No. 15017. Ottawa, Ontario. 166 pp.

Nemeth, A. and R. Herrera. 1982.
Earthworm populations in a tropical rain forest. Pedobiologia. 23: 437-443.

Neuhauser, E.F., D.L. Kaplan and M.R. Malecki. 1980.
Materials supporting weight gain by the earthworm *Eisenia foetida* in waste conversion systems. Agric. Wastes. 2: 43-60.

Perel', T.S. 1977.
"Differences in Lumbricid organization connected with ecological properties." In. Soil Organisms as Components of Ecosystems. Proc. 6th Int. Coll. Soil Zool. U. Lohm and T. Persson (eds.) Ecol. Bull. (Stockholm) 25: 122-132.

Phillipson, J., R. Abel, J. Steel and S. Woodell. 1976.
Earthworms and the factors governing their distribution in an English beechwood. Pedobiologia. 16:258-285.

Pietz, R.I., J.R. Peterson, J.E. Prater and D.R. Zenz. 1984.
Metals concentrations in earthworms from sewage sludge-amended soils at a strip mine reclamation site. J. Environ. Qual. 13(4): 651-654.

Rai, D. and L.E. Eary. 1986.
"Solubility - controlling solids of Cr(III) and Cr(III)/Cr(VI) transformation reactions." In. Proceedings, Chromium symposium 1986: an update. D.M. Serrone (ed.) Industrial Health Foundation, Inc. Pittsburg PA. pp. 331-341.

Rai, D. B.M. Sass and D.A. Moore. 1987.
Chromium(III) hydrolysis constants and solubility of chromium(III) hydroxide. Inorg. Chem. 26: 345 - 349

Rai, D., J.M. Zachara, L.E. Eary, C.C. Ainsworth, J.E. Amonette, C.E. Cowan, R.W. Szelmeczka, C.T. Resch, R.L. Schmidt, D.C. Girvin and S.C. Smith. 1988.
Chromium reactions in geologic materials. Electric Power Research Institute, Palo Alto, CA. EPRI report EA-5741. 291 pp.

Rai, D., J.M. Zachara, L.E. Eary, D.G. Girven, D.A. Moore, T. Resch, B.M. Sass and R. Schmidt. 1986.
Chemical attenuation studies: geochemical reactions of Cr(III) and Cr(VI). Electric Power Research Institute. Palo Alto, CA. 280 pp.

Raymont, J.E. and J. Shields. 1963.
Toxicity of Cu and Cr in the marine environment. Air Water Pollut. 7: 435-443.

Reichle, D.E. 1977.
"The role of soil invertebrates in nutrient cycling." In. Soil organisms as components of ecosystems. Proc. 6th Int. Coll. Soil Zool. U. Lohm and T. Persson, (eds.) Ecol. Bull (Stockholm) 25: 145-156.

Reisenauer, H.M. 1982.
"Chromium" In. Methods of Soil Analysis Part 2: chemical and microbiological properties. 2nd edition. No. 9, Agronomy Series. A.L. Page (ed.) Am. Soc. of Agron. Inc., Madison, WI. pp. 337-346.

Reynolds, J.W. 1977.
The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Royal Ontario Museum, Misc. Publ. in the Life Sci. pp. 108-111.

Rhee, J.A. van. 1975.
"Copper contamination effects on earthworms by disposal of pig waste in pastures." In. Progress in Soil Zoology. Proc. 5th Int'l. Colloq. Soil Zool., Prague. J. Vanek (ed.) pp. 451-457.

Richards, K.S. and M.P. Ireland. 1978.
Glycogen - lead relationship in the earthworm *Dendrobaena rubida* from a heavy metal site. Histochemistry. 56: 55-64.

Ross, D.S., R.E. Sjogren and R.J. Bartlett. 1981.
Behavior of chromium in soils: IV. toxicity to microorganisms. J. Environ. Qual. 10(2): 145-148.

Rouelle, J. 1983.
"Introduction of amoebae and *Rhizobium japonicum* into the gut of *Eisenia fetida* (Sav.) and *Lumbricus terrestris L.*" In. Earthworm ecology: from Darwin to vermiculture. J.E. Satchell (ed.) Chapman and Hall, London, U.K. pp. 375-381.

Rushton, S.P. 1986.
Development of earthworm populations on pasture land reclaimed from open-cast coal mining. Pedobiologia. 29:27-32.

Satchell, J.E. 1983.
"Earthworm microbiology." In. Earthworm ecology: from Darwin to vermiculture. J.E. Satchell (ed.) Chapman and Hall, London, U.K. pp. 351-364.

Scheu, S. 1987a.
Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae). Biol. Fertil. Soils. 5: 230-234.

Scheu, S. 1987b.
The influence of earthworms (Lumbricidae) on the nitrogen dynamics in the soil litter system of a deciduous forest. Oecologia (Berl.) 72: 197-201.

Scheu, S. 1987c.
The role of substrate feeding earthworms (Lumbricidae) for bioturbation in a beechwood. Oecologia (Berl.). 72: 192-196.

Shaw, C. 1984.
Genetic alterations to artificial soils by earthworms. M.Sc. thesis, Dept. of Soil Science, University of Alberta. 320 pp.

Shaw, C. and S. Pawluk. 1986a.
Faecal microbiology of *Octolasion tyrtaeum, Aporrectodea turgida* and *Lumbricus terrestris* and its relation to the carbon budgets of three artificial soils. Pedobiologia. 29: 377-389.

Shaw, C. and S. Pawluk. 1986b.
The development of soil structure by *Octalasion tytraeum, Aporrectodea turgida* and *Lumbricus terrestris* in parent materials belonging to different textural classes. Pedobiologia. 29: 327-339.

Shen, S.M., G. Pruden and D.S. Jenkinson. 1984.
Mineralization and immobilization of nitrogen in fumigated soil and the measurement of microbial biomass nitrogen. Soil Biol. Biochem. 16(5):437-444.

Shewry, P.R. and P.J. Peterson. 1974.
The uptake and transport of chromium by barley seedlings (*Hordeum vulgare L.*). J. Exptl. Bot. 25(87): 785-797.

Snedecor, G.W. and W.G. Cochran. 1980.
Statistical Methods. Seventh Edition. Iowa State University Press. Ames, IA. 507 pp.

Southwell, L.T. and J.D. Maier 1981.
The survival and growth of the earthworm *Eisenia foetida* (Lumbricidae: Oligochaeta) in alkaline residues with bauxite refining process. Pedobiologia. 22:42-52.

Spiers, G.A., D.Gagnon, G.E. Nason, E.C. Packee and J.D. Lousier. 1986.
Effects and importance of indigenous earthworms on decomposition and nutrient cycling in coastal forest ecosystems. Can. J. For. Res. 16:983-989.

Stephenson, J. 1930.
The Oligocheata. Clarendon Press, Oxford. 482 pp.

Stollenwerk, K.G. and D.B. Grove. 1985a.
Adsorption and desorption of hexavalent chromium in an alluvial aquifer near Telluride, Colorado. J. Environ. Qual. 14(1): 150-155.

Tomlin, A.D. 1983.
"The earthworm bait market in North America." In. Earthworm Ecology: from Darwin to vermiculture. J.E. Satchell (ed.) Chapman and Hall, London, U.K. pp. 331-338.

Turner, M.A. and R.H. Rust. 1971.
Effects of Cr on growth and mineral nutrition of soybeans. SSSAP. 35: 755-758.

Van Hook, R.I. 1974.
Cadmium, lead and zinc distributions between earthworms and soils: potential for biological accumulation. B. Envtl. Cont. & Tox. 12: 509-511.

Voroney, R.P. and E.A. Paul. 1984.
Determination of k_C and k_N in situ for calibration of the chloroform fumigation-incubation method. Soil Biol. Biochem. 16(1):9-14.

Wielgus-Serafinska, E. and E. Kawka. 1976.
Accumulation and localization of lead in *Eisenia foetida* (Oligochaeta) tissues. Folia Histochemica et Cytochemica. 14: 315-320.

Zachara, J.M., D.C. Girvin, R.L. Schmidt and C.T. Resch. 1987.
Chromate adsorption on amorphous iron oxyhydroxide in the presence of major groundwater ions. Environ. Sci. Tech. 21: 589-594.

Zachmann, J.E., D.R. Linden and C.E. Clapp. 1987.
Macroporous infiltration and redistribution as affected by earthworms, tillage and residue. SSSAJ. 51: 1580-1586.

Zibilske, L.M. and G.H. Wagner. 1982.
Bacterial growth and fungal genera distribution in soil amended with sewage sludge containing cadmium, chromium, and copper. Soil Sci. 134(6): 364-370.

APPENDIX: ADDRESSES FOR ANALYSES OR ASSISTANCE EXTERNAL TO UNIVERSITY OF ALBERTA.

1. Source of *Eisenia fetida* individuals: Altwin Distributors, P.O. Box 1448, Medicine Hat, AB, T1A 7N4.

2. Confirmation of earthworm species identification: Dr. V. Marshall, Pacific Forestry Centre, 506 West Burnside Rd., Victoria, BC, V8Z 1M5.

3. INAA analysis: Nuclear Activation Services Ltd., 1280 Main St. West, Hamilton, ON, L8S 4K1.

4. Data analyses performed using PC-SAS Version 6. © SAS Institute, Cary, NC, U. S. A.